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# CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

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# INDIVIDUAL PROTECTION TESTING
## (TASK 5 - QUALITATIVE FIT TEST SIMULANTS)

M. R. Kuhlman
K. C. Hofacre
J. D. Rench

BATTELLE MEMORIAL INSTITUTE
Columbus, OH 43201-2693

P. D. Gardner

PHYSICAL PROTECTION DIRECTORATE

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Kuhlman, M. R.; Hofacre, K. C.; and Rench, J. D. (Battelle); and Gardner, P. D. (CRDEC)

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A group of potential replacement qualitative fit test simulants was examined for their effectiveness and compared against the currently used compound, isoamyl acetate (IAA) or banana oil. The literature on odorant compounds was assessed to identify compounds which might be useful, and the toxicological literature was used to screen those for safe compounds. A set of four compounds: nonanoic acid, skatole, t-butyl mercaptan, and methyl salicylate was chosen for odor threshold measurement, along with IAA. The odor threshold measurements were made using a panel of volunteers and a novel odor presentation technique in a forced-choice triangle, ascending odor design. The results of that testing eliminated nonanoic acid and methyl salicylate from further consideration. Simultaneous qualitative and quantitative fit testing was performed on a panel of subjects using each of the odorants: IAA, t-butyl mercaptan, and skatole. The test results were as consistent as can be expected when using a panel to detect odors. The data obtained indicate that t-butyl mercaptan could be used as a qualitative fit test challenge to achieve mask protection factors significantly higher than those achievable with IAA.

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The work described in this report was authorized under Contract No. DAAA15-85-D-0010. This work was started in February 1987 and completed in March 1989.

In conducting the research described in this report, the investigators adhered to Army Regulation 70-25, Research and Development--Use of Volunteers as Subjects of Research, dated 3 July 1974, as promulgated by the Office of The Surgeon General, Department of the Army.

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## INDIVIDUAL PROTECTION TESTING
## (TASK 5--QUALITATIVE FIT TEST SIMULANTS)

1.      INTRODUCTION

The protection which any mask affords the wearer depends
heavily on the quality of fit achieved. Small adjustments of the fit of
a properly sized mask can result in significant changes in mask perfor-
mance. For the typical user, however, the appearance and feel of the fit
provide insufficient information to optimize mask performance. To
provide the soldier with an indication of the quality of fit he has
achieved with his mask, the U.S. Army presently uses banana oil, or
isoamyl acetate (IAA), as an odorant challenge. This vapor is effi-
ciently absorbed by the charcoal filter system of the mask, so any odor
detected by the masked individual indicates a leakage pathway exists.
Usually, such leakage paths can be greatly reduced by adjusting the mask
harness until the odor is no longer detected.

The protection factor (PF) of a mask is defined by the ratio of
the challenge concentration outside the mask to that inside the mask. If
the soldier's sense of smell serves as the detector inside the mask, then
the PF can only be reliably improved to the point where the odor inside
the mask is no longer detectable. For a given odorant challenge
concentration, therefore, a lower odor detection threshold will enable
the soldier to achieve greater protection using the qualitative fit test.

Objective.

The objective of this task is to identify new nonhazardous
chemicals which have lower odor/irritant threshold levels than IAA and
can be safely used to qualitatively evaluate the fit of various military
protective mask systems. Ideally, the simulant will also be capable of
being used as a training agent in the field.

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## 2.    APPROACH

The approach followed to meet the objective of this task consisted of several steps.  First was the development of a set of criteria to be used in selecting candidate odorant compounds, and the use of available literature to select a set of candidates to be evaluated experimentally.  The experimental evaluation of the candidate compounds consisted of determining the odor threshold for the candidates and using those results to select the most promising compounds.  Finally, the selected compounds were used in conjunction with quantitative fit testing using a modified M17 respirator to determine the correlation between the qualitative and quantitative fit test results.  Each of these steps is described in more detail in the following sections.

## 2.1    Odorant Selection Criteria.

The selection of candidate compounds for further investigation as potential simulants was accomplished by first specifying the criteria which would be used to screen the candidates.  This list of criteria can be subdivided into three categories:

### Effectiveness Criteria

- Ratio of vapor pressure to odor/irritant (O/I) 100 percent recognition threshold will exceed 10,000. (Required)

- Charcoal adsorption capacity will exceed 3 mg/g. (Required)

- O/I 100 percent recognition threshold will be less than 0.23 ppm.  (Desired)

- Vapor pressure should exceed 0.5 mmHg at 20°C. (Desired)

- Punitive or malodorous nature.  (Desired)

- Different chemical structures for O/I candidates. (Desired)

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## Practicality Criteria

- Costs for procurement or synthesis to be less than $500/1. (Required)

- Boiling point to exceed 60°C. (Required)

- Stability/reactivity to be such that a one-year storage period at ambient temperature is possible. (Desired)

## Safety Criteria

- Flash point must exceed 40°C. (Required)

- Noncarcinogenic. (Required)

- Ratio of TLV (if one exists) to O/I 100 percent recognition threshold will exceed 1000. (Required)

The principles behind the effectiveness criteria are the following. A lower limit of 10,000 was chosen for the odor index (ratio of vapor pressure to odor/irritant 100 percent recognition threshold). Given this value, with a challenge of the room temperature vapor pressure of the simulant, a mask fit factor of 10,000 would be detectable for the panel whose response determined the odor threshold for the compound. An individual whose response differed by a factor of 10 (such differences are not uncommon) from that of the panel could still detect a fit factor of 1000, which is the target value for the selected simulant. Below this limit on the odor index the compound may not be practical for testing purposes.

It was determined that a charcoal adsorption capacity of at least 3 mg/g would be required in order to avoid filter breakthrough of the compound within 10 minutes for fit testing under anticipated challenge concentrations and breathing rates. The 100 percent recognition threshold for isoamyl acetate (0.23 ppm) was used as the upper limit for the candidates' detectability during the screening process, since the objective is to find a simulant better than isoamyl acetate. A lower limit of 0.5 mmHg (@20°C) was chosen for the vapor pressure to reduce

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measurement problems which might arise from inadequate instrument sensitivity.

Punitive or malodorous compounds were considered to be desirable because they would elicit a physical reaction upon detection. Published studies of odor thresholds of compounds have indicated that a subject will detect a pleasant or neutral compound with much less certainty than a malodorous one. This results, frequently, in lower reported odor thresholds for less pleasant smelling compounds. Also, compounds which were similar in structure or chemical class to another (better) candidate were eliminated.

The principles behind the practicality criteria, cost and storageability, are self-explanatory. The boiling point should exceed 60°C in order to eliminate the possibility that the compound (in liquid form) could change to a gas during storage under hot conditions and result in seal failure and leakage.

The reasons for the safety criteria regarding flash point and noncarcinogenicity are self-explanatory. The ratio of the threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists (ACGIH) to odor recognition threshold should exceed 1000 so that the individual wearing the mask can be exposed safely to a high enough challenge concentration of the compound with an acceptable margin of safety.

2.2     Candidate Odorants.

The initial list of compounds to be evaluated relative to the above criteria was compiled from tabulations found in the litera-ture.[1,2] Additional compounds were placed on the initial list presented in Appendix A based on gas odorization literature or recommendations by experts in the field. The compound classes in this initial

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li.t include principally mercaptans, esters, ketones, sulfides, acrylates, amines, ethers, aldehydes, acids, alcohols, and acetates.

Several compounds were removed from the initial list because they were carcinogenic or suspected carcinogens[3]. Over half of the compounds were removed from further consideration because their odor thresholds were greater than 0.23 ppm, or because their odor indexes were less than 10,000. Approximately 30 compounds were deleted because of an unfavorable boiling point or flash point.

Finally, those compounds having weak or pleasant odors which also had insufficient information available were eliminated. Compounds which were similar in structure or chemical class to another candidate were also eliminated at this point. This last step reduced the list by a total of 52 compounds.

Table 1 lists the 15 candidates which remained and the known values for properties for which selection criteria were identified above. Although odor threshold values are not identified for thiophane or bromoacetone, they are undoubtedly detectable at very low concentrations. Thiophane is used for gas odorization in industry[4]. Bromoacetone has been used (in Germany) as a fit test simulant, but no literature describing its use was found.

2.2.1    Toxicity Screening of Candidate Compounds.

With the list of candidate compounds reduced to those in Table 1, standard toxicology reference literature was reviewed to indicate which, if any, of the compounds would be unsuitable for human exposures in testing. Any positive evidence of carcinogenicity, mutagenicity, or reproductive toxicity was deemed sufficient for elimination of the compound from further consideration for use. Also, if any U.S. or European occupational exposure guidelines were not compatible

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## TABLE 1. CANDIDATE QUALITATIVE FIT TEST COMPOUNDS AND PHYSICAL PROPERTIES

| Compound [CAS Registry No.] | Odor Recognition Threshold, ppb (50%, unless noted otherwise) | Odor Index | Threshold Limit Value, ppm | Boiling Point, C | Flash Point, C | Punitive/ Malodorous |
|---|---|---|---|---|---|---|
| n-butyl sulfide-$C_8H_{18}S$ [544-40-1] | 2 (100%) | 658,000 | -- | 182 | -- | Unpleasant |
| n-butyric acid-$C_4H_8O_2$ [107-92-6] | 20 | 50,000 | 2.5 | 164 | 77 | Unpleasant |
| Cinnamaldehyde-$C_9H_8O$ [104-55-2] | 2.5 | 53,000 | -- | 246 | -- | -- |
| Methyl salicylate-$C_8H_8O_3$ [119-36-8] | 0.58 | 113,400 | -- | 224 | 99 | -- |
| 2-octanol-$C_8H_{18}O$ [123-96-6] | 0.26 | 506,000 | 20 | 179 | 60 | Unpleasant |
| Propionic acid-$C_3H_6O_2$ [79-09-4] | 34 (100%) | 112,200 | 15 | 141 | 57 | Pungent |
| Pyridine-$C_5H_5N$ [110-86-1] | 100 | 184,200 | 5 | 116 | 75 | Unpleasant |
| Thymol-$C_{10}H_{14}O$ [89-83-8] | 0.85 | 155,000 | -- | 233 | -- | Pungent (crystals) |
| Sulfur dichloride-$SCl_2$ [10545-99-0] | 1 (100%) | -- | -- | 59 | -- | Irritant |
| Thiophane-$C_4H_8S$ [110-01-0] | -- | -- | -- | 120 | -- | Unpleasant |
| Bromoacetone-$C_3H_5BrO$ [598-31-2] | -- | -- | -- | 137 | -- | Irritant |
| tert-Butyl mercaptan-$C_4H_{10}S$ [75-66-1] | 0.08 | -- | 0.5 | 64 | -- | Unpleasant (very stable) |
| Skatole-$C_9H_9N$ [83-34-1] | 0.075 | 30,000 | -- | 266 | -- | Unpleasant (crystals) |
| Valeric acid-$C_5H_{10}O_2$ [109-52-4] | 0.6 | 329,000 | -- | 187 | -- | Unpleasant |
| Pelargonic acid-$C_9H_{18}O_2$ [112-05-0] | 0.84 | 164,000 | -- | 258 | -- | Irritant |

with the envisioned testing, the candidate was rejected. The results of the literature assessment are summarized in Table 2.

Bromoacetone was eliminated because of its high acute toxicity. The candidate, 2-octanol, was eliminated from further consideration here because of its inclusion in the National Toxicology Program, despite the absence of evidence of toxicity at this time. Thiophane was removed from the potential candidate list because of the complete absence of information regarding the toxicity of this compound. The highly corrosive nature of sulfur dichloride resulted in the rejection of this compound for use in human exposures. The remaining candidates: t-butyl mercaptan, n-butyl sulfide, nonanoic acid, pentanoic acid, and skatole were judged to be acceptable for testing in this project, but were too numerous for all to be retained. After discussion between project staff and the CRDEC project officer, it was decided that the following candidates be included in the odor threshold determination portion of this study: t-butyl mercaptan, methyl salicylate, nonanoic acid, and skatole. Isoamyl acetate was also retained to provide a baseline against which the potential replacement compounds could be evaluated.

A review of the toxicity of the five compounds which were used in odor threshold testing is contained in Appendix B. That information was prepared in support of the human use protocol which required approvals before testing with human subjects could be performed.

2.2.2    Preliminary Assessment of Candidate Interaction
         with Respirators and Charcoal Cartridge.

The possibility of respirator breakthrough was assessed for each of the new compounds using a computer program which combined the Dubinin-Raduschkevich isotherm with the Wheeler-Robbell bed permeation model[13]. For these calculations, it was assumed that the challenge concentration of each compound was 100 ppm, the weight of the carbon bed was 105 g, and the air flow rate through the bed was a constant 20 lpm.

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TABLE 2.  RESULTS OF TOXICOLOGICAL SCREENING OF CANDIDATE CHEMICAL SUBSTANCES

| Chemical (Registry No.) | Carcinogenicity | Reproductive Toxicity | Mutagenicity | Toxicity | Occupational Guidelines |
|---|---|---|---|---|---|
| Bromoacetone (CAS: 598-31-2) | NPE | NPE | NPE | High acute toxicity in humans (LCLo = 572 ppm/10 Min in inhalation, human) (RTECS) | None |
| t-butyl mercaptan (CAS: 75-66-1) | NPE | NPE | NPE | Slight acute toxicity (LD50 = 4,729 mg/kg: oral, rat) (RTECS); practically non-toxic (LC50 = 22,000 ppm [4 hr]: inhalation, rat) (Patty's); unspecified irritation at 84 mg (eye, rabbit) (RTECS) | None |
| n-butyl sulfide (CAS: 544-40-1) | NPE | NPE | NPE | Slight acute toxicity (LD50 = 2,220 mg/kg: oral, rat) (RTECS); moderate acute toxicity (LCL = 1,800 mg/m³: inhalation, mouse) moderate irritant (skin, rabbit) (RTECS) | None |

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TABLE 2. (Continued)

| Chemical (Registry No.) | Carcinogenicity | Reproductive Toxicity | Mutagenicity | Toxicity | Occupational Guidelines |
|---|---|---|---|---|---|
| n-butyric acid (CAS: 107-92-6) | NPE | NPE | Two positive in vitro studies (RTECS); NTP testing in progress | Slight acute toxicity (LD50 = 2,940 mg/kg: oral, rat) (Sax) Severe irritant (10 mg/24 H: skin, rabbit); Moderate irritant (500 mg open: skin, rabbit) (Sax) | None |
| Cinnamaldehyde (CAS: 104-55-2) | One study - equivocal evidence (TDLo = 800 mg/kg 8W-I: intraperitoneal, mouse) (RTECS); NTP testing underway | NTP short-term in vivo testing underway | NPE | Slight acute toxicity (LD50 = 2,220 mg/kg: oral, rat) (RTECS) Severe irritant (40 mg/48 H: skin, human) | None |
| Methyl salicylate (CAS: 119-36-8) | One study - equivocal evidence (TDLo = 2,400 mg/kg 8W-I: intraperitoneal, mouse) (RTECS) | Two positive teratogenicity assays in mice, rabbits and rats (Schardein) | NPE | Slight acute toxicity (LD50 = 887 mg/kg (oral, rat) (RTECS) Severe irritant (500 mg/24 H: eye, rabbit) (RTECS); Severe irritant (500 mg open: skin, guinea pig) (Sax) | None |

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TABLE 2.  (Continued)

| Chemical (Registry No.) | Carcinogenicity | Reproductive Toxicity | Mutagenicity | Toxicity | Occupational Guidelines |
|---|---|---|---|---|---|
| Nonanoic acid (CAS: 112-05-0) | NPE | NPE | NPE | Slight acute toxicity (LD50 = 3,200 mg/kg: oral, rat) (RTECS); Severe irritant (91 mg: eye, rabbit) (RTECS); Severe irritant (500 mg: skin, guinea pig) (RTECS) | None |
| 2-octanol (CAS: 123-96-6) | NPE | NPE | Selected for mutagenicity testing by NTP in 1986 | ND | None |
| Pentanoic acid (CAS: 109-52-4) | NPE | NPE | NPE | LD50 = 1,290 mg/kg (intravenous, mouse) (RTECS); LD50 = 3,590 mg/kg (subcutaneous, mouse) (RTECS); LD50 = 700 mg/kg (dermal, rabbit) (Sax) | None |
| Propionic acid (CAS: 79-09-4) | NPE | NPE | NPE | Slight acute toxicity (LD50 = 2,500 mg/kg: oral, rat) | ACGIH: TLV (TWA)= 10 ppm (30 mg/m$^3$) TLV (STEL) = 15 ppm (45 mg/m$^3$) |

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TABLE 2. (Continued)

| Chemical (Registry No.) | Carcinogenicity | Reproductive Toxicity | Mutagenicity | Toxicity | Occupational Guidelines |
|---|---|---|---|---|---|
| Propionic acid (CAS: 79-09-4) (Con't.) | | | | (RTECS) Severe irritant (990 ug: eye, rabbit) (Sax) | Designed to prevent irritation of eyes and respiratory passages |
| Pyridine (CAS: 110-86-1) | Carcinogenicity bioassays in progress by NTP | Positive tera-togeneticity assays in chickens (Shepard) | Positive assay (Salmonella typhimurium) (RTECS)  Positive response for sister chromatid exchange (GENETOX) | Slight acute toxicity LD$_{50}$ = 891 mg/kg: oral, rat) (RTECS)  Mild irritant (10 mg/24 hrs: skin, rabbit) (RTECS); severe irritant (2 mg: eye, rabbit) (RTECS) | ACGIH: TLV (TWA) = 5 ppm (15 mg/m3) TLV (STEL) = 10 ppm (30 mg/m3)  OSHA: PEL = 5 ppm  Values set to prevent systemic effects from exposure; skin absorption is possible |
| Skatole (CAS: 83-34-1) | NPE | NPE | NPE | LDLo = 1,000 mg/kg (subcu-taneous, frog) (RTECS) | None |

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## TABLE 2. (Continued)

| Chemical (Registry No.) | Carcinogenicity | Reproductive Toxicity | Mutagenicity | Toxicity | Occupational Guidelines |
|---|---|---|---|---|---|
| Sulfur dichloride (CAS: 10545-99-0) | NPE | NPE | NPE | No acute toxicity data; Highly irritative to skin, eyes, mucous membranes; corrosive to tissue (Sax) | None |
| Thiophane (CAS: 110-01-0) | NPE | NPE | NPE | ND | None |
| Thymol (CAS: 89-83-3) | One study - equivocal evidence (TDLo = 1,200 mg/kg 8W-I: intraperitoneal, mouse) (RTECS) | One positive study (TDLo = 20 mg/kg (4D pre subcutaneous, rat) (RTECS) | One positive assay Saccharomyces cerevisiae (RTECS) | Slight acute toxicity (LD50 = 980 mg/kg oral, rat) (RTECS) | None |

ACGIH = American Conference of Governmental Industrial Hygienists
LCLo = Lowest concentration of a substance in air that, after exposure, produces any toxic, tumorigenic, or reproductive effect
LDLo = Lowest dose of a substance introduced by a route other than inhalation over any period of time and reported to have caused death in humans or animals
LD50 = Lethal dose for 50 percent of the test organisms
ND = No data found in source documents
NPE = No positive evidence found in source documents
NTP = National Toxicology Program
PEL = Permissible exposure limit
TDLo = The lowest dose of a substance administered that produces tumorigenic or reproductive effects in test organisms
TLV (STEL) = Threshold limit value: short-term exposure limit
TLV (TWA) = Threshold limit value: time-weighted average

Sources: ACGIH (Reference 5); GENETOX (Reference 6); Schardein (Reference 7); RTECS (Reference 8); Sax (Reference 9); Shephard (Reference 10); NTP (Reference 11); Patty's Industrial Hygiene and Toxicology (Reference 12).

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These were intended to be worst-case assumptions. The carbon adsorption values and breakthrough times for the compounds are given below:

| Compound | Carbon Adsorption, g sorbate g carbon | Breakthrough Time, min |
|---|---|---|
| Nonanoic acid | 0.214 | 1673.7 |
| Skatole | 0.231 | 2080.6 |
| t-butyl mercaptan | 0.163 | 78.9 |
| Methyl salicylate | 0.219 | 1702.2 |

The carbon adsorption values were large enough that the time required for each compound's breakthrough to reach its respective detection limit was well over 10 minutes, even at a very high assumed challenge concentration. Therefore, none of the four compounds was eliminated due to the possibility of respirator breakthrough during fit testing.

3.    ODOR THRESHOLD DETERMINATION

3.1    Objective.

The objective of this portion of the project was the measurement of the odor thresholds for IAA and for the candidate compounds selected above. There are several reasons for performing such measurements. Reported threshold concentrations for a given compound may vary greatly, due to different measurement methods and different modes of presenting the compound to the panelists. This is to say nothing of the effects on reported threshold concentrations caused by different numbers of panelists and panel composition. Threshold values obtained using a small panel of trained experts will yield a different value than those obtained with a large group of naive subjects. Smoking is a factor which has been found, in some cases, to reduce the olfactory sensitivity of subjects.[14,15] Frequently, smokers are excluded from panels used to

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determine odor thresholds which are reported. It would clearly not be acceptable for our purposes here to omit the threshold for smokers from consideration. Finally, the manner in which an odor is presented to a panelist can influence the level at which it is detected. This is a fairly obvious factor when odors are presented in flasks of solutions as opposed to a flowing gas stream. But less obvious factors, such as air temperature, relative humidity, and velocity can influence odor detection.[15] The procedure adopted for these tests is described below. This procedure attempts to duplicate the conditions of respirator testing insofar as is practicable.

3.2    Experimental Procedure.

Odor threshold determinations of the candidate compounds were performed using a panel of 19 subjects, each of whom was tested in an identical fashion in accordance with the test protocol described below.

The number and distribution of subjects tested is presented in Table 3. Females were excluded from the subject pool because of the need to obtain approval of the human use protocols by both Battelle and U.S. Army review boards. At the outset of this project, the Battelle review board expressed reservations regarding any use of females of child-bearing age. It was originally planned that at least 20 male subjects between the ages of 18 and 35 years be tested. However, due to a dearth of interested subjects only 19 males were tested. Furthermore, with the lack of subjects, we could not meet our planned distribution of 50 percent smokers and 33 percent blacks, which was intended to reflect the U.S. Army population.

TABLE 3. MATRIX OF SUBJECTS USED IN ODOR
THRESHOLD TESTING

|       | Nonsmoker | Smoker | Total |
|-------|-----------|--------|-------|
| White | 9         | 7      | 16    |
| Black | 2         | 1      | 3     |
| TOTAL | 11        | 8      | 19    |

All panelists were screened for olfactory dysfunction prior to their inclusion in the study. Because IAA serves as the standard against which the candidate compounds are to be judged, all subjects were tested to ensure that they were able to detect this odor at relatively low levels. The subjects were individually presented with three stoppered flasks and requested to identify the flask containing an odor. Two of the flasks contained odor-free water, and the third contained 1.25 ppm IAA in water. The subjects identified the correct flask with varying degrees of certainty, but all identified the proper flask.

3.2.1    Test Method.

A wide variety of methods have been employed in other studies to present the odorant samples to panelists. Different methods usually lead to different levels of dilution of sample between sample container and olfactory receptors. Factors such as air velocity, temperature, and relative humidity are also thought to influence measured odor threshold values[16]. It was therefore considered prudent to conduct odor threshold determinations under conditions similar to qualitative fit testing. Hence, for this effort, thresholds were measured while a subject wore a modified M17 respirator. The respirator was modified to permit the wearer to inhale directly from a manifold carrying the test gas stream. This inhaled air was directed into the respirator, passing through the charcoal filters without contacting the filter material.

This permits the gas stream to pass over the face of the subject and mask surface so that the temperature and humidity of the gas stream are similar to those during respirator wear, and so any interactions of the vapors with the mask components, or subject's face are included. While this was not a perfect simulation of the field situation, it should, nevertheless, provide measurements which will more closely mimic field performance.

The test protocol followed for determining odor threshold values of each candidate compound is a forced-choice triangle design[16,17], which presents the odor concentrations to the subject in an ascending order. Each level of concentration used in the testing differs by a factor of three from the nearest concentration. For example, if the estimated odor threshold concentration of a compound is X, then, ideally, the concentrations presented to the panelist would be X/27, X/9, X/3, X, 3X, 9X, 27X. This set of concentrations provides nearly three orders of magnitude range in concentration. Each concentration was targeted to be within 10 percent of its nominal value because concentrations that differ by less than 10 percent are typically not well differentiated by subjects.[18]

The forced-choice triangle experimental design presents the subject with three sample gas streams at each concentration level, with two of the three being blanks--in this case, odor-free air. For each set of three, the coding is randomized so that the subject cannot rely on visual cues or order of presentation to determine which of the three samples contains the odorant vapor. After the subject has had a chance to smell the three samples, he is required to select (or guess) which of the samples contained the odorant, even if he has not detected an odor. The subject is free to repeat any of the samples within a set of three. This sampling/decision process is repeated at progressively higher concentration levels until the subject has correctly identified the odor stream at three consecutive levels. Once three consecutive correct selections are made, testing for this compound ceases. It is important

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to note that testing starts at the lowest concentration level because the use of an ascending order of concentration eliminates the possibility of having a near threshold concentration presented to a subject who is in a state of olfactory fatigue from having just received a much higher concentration.  It is also to be noted that, by forcing the subject to choose one of the three gas streams, even when unsure of the odor's presence, more reproducible results are obtained in testing with panels. The variability in confidence of the panelists is removed from the factors affecting the indicated threshold of detection.  The threshold concentration for an individual is then defined to be between the highest concentration at which an incorrect selection was made by the panelist and the lowest of three consecutive concentrations at which the odorant gas stream was correctly identified.

### 3.2.2    Test Equipment.

The principal equipment used in the odor threshold testing consists of the challenge generators, the serial dilution apparatus, the odor exposure chamber and mask, and the gas chromatograph used for measuring vapor concentrations.  Each of these items are described in the following paragraphs.

Generation of odor was accomplished by use of a bubbler apparatus, which contained either the pure odorant liquid or an aqueous solution of the compound.  Clean air flowed through the capillary which was submerged in the liquid, bubbled up through the liquid, and was carried to the dilution system.

When air is bubbled through the capillary tubing in the liquid, the equilibrium concentration of odorant is approached in the vapor phase.  The concentration of odorant in the vapor phase depends upon temperature, the pure component vapor pressure, and the composition of liquid if a solution is used.  The bubbler temperature was maintained by a constant temperature water bath to control the equilibrium vapor concentration.  With this steady concentration, the amount of odorant

(mass flow rate) delivered to the diluter was controlled by varying the
air flow rate through the bubbler, which is regulated by establishing the
appropriate pressure drop across the capillary.

The compound, t-butyl mercaptan, did not lend itself to
bubbling through the pure component liquid because the resulting
concentration of odorant in the vapor could not be readily diluted below
its threshold concentration. The melting point of skatole is 95°C, and
at this temperature, it was doubtful that adequate dilution of the vapor
could be achieved. For this compound therefore, water was used as a
solvent since an organic solvent may be detectable before the odorous
compound. As was the case with bubbling through the pure component,
bubbling through a solution produces an equilibrium concentration of
odorant in the vapor phase. However, this equilibrium concentration
depends upon the molar concentration of odorant in the liquid phase. A
saturated solution of the sparingly soluble skatole was used, and a very
dilute solution of t-butyl mercaptan was used. The final concentration
in vapor phase was regulated by adjustment of the air flow rate through
the capillary. Bubbler operating parameters are summarized in Table 4.

Each day of testing these conditions were set to produce the
same amount of odorant for each subject tested. Only in the case of tBM
could the liquid composition change and, thus, the vapor phase concentra-
tion change. So, when generating tBM vapor, the solution was frequently
changed in order to keep it fresh. At the start of each test day a new
supply of test solution was made from the stock. Also, not more than
four subjects were tested without replacing the test solution in the
bubbler. Calculations determined that tBM concentration in solution
would not change appreciably during odor generation for testing four
subjects.

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TABLE 4.  ODORANT BUBBLER OPERATING PARAMETERS
FOR ODOR THRESHOLD TESTING

| Odorant | Bubbler Contents | Bubbler Temp, C | Bubbler Air Flow Rate, $cm^3/min$ |
|---------|------------------|-----------------|-----------------------------------|
| t-Butyl mercaptan (tBM) | $H_2O$ solution[a] | 40 | 10 |
| Isoamyl acetate (IAA) | Pure component | 25 | 32 |
| Methyl salicate (MeS) | Pure component | 25 | 60 |
| Nonanoic acid | Pure component | 40 | 125 |
| 3-Methyl indole | $H_2O$ solution[b] | 40 | 132 |

(a)  Stock solution of 1 cc from tBM in 1000 cc $H_2O$.  5 ml stock
     solution further diluted in 100 cc $H_2O$.
(b)  A saturated solution of skatole in $H_2O$ with excess skatole.


The odor exiting the bubbler was immediately diluted with 1 lpm
of clean air which, in turn, carried the odor stream into the diluter.
This initial dilution aided in preventing recondensation of the odorant
compound, in transit to the dilution apparatus.

The diluter makes successive 2:1 dilutions of clean air:odor-
laden air to reduce the odor concentration by a factor of three at each
of up to seven stages.  Proper dilution is ensured at each stage by
adjustment of mixer flow meters to the desired values.  Following a
series of 2:1 dilutions, 1 lpm of the odor stream is further diluted with
35 lpm of clean air to provide an ample supply of air for subjects to
breathe.

The concentration of odorant delivered to a subject was
controlled by selecting the amount of dilution supplied by a serial
dilution apparatus.  As the number of dilution stages decreased from

eight to two, the output concentration increased. A control box with a selector dial allowed for switching from one stage to another by activating solenoid valves. These solenoid valves controlled the path of odor-laden and dilution air, thus, controlling the extent of dilution.

A few additional features were necessary for operation of the dilution apparatus. A cartridge containing activated charcoal was affixed to the diluter so that excess air streams containing odor could be dumped to the atmosphere (via an exhaust hood) after passing through this scrubber. Also, when the odor stream was not delivered to the subject, it passed through the scrubber. Finally, a clean air system was added so that all lines could be purged before a new odor was generated, thereby eliminating any carryover from the previous test.

The serial diluting system used to vary the odor concentration by factors of three between successive stages was calibrated by using "P10" gas (a mixture of 10 percent methane and 90 percent argon) as the test gas. A flow rate of 1 lpm of P10 gas was delivered to the diluter inlet for dilution. Proper dilution (2 lpm of clean air and 1 lpm of odorant containing air) was obtained by adjusting the mixer rotameters at each stage of the apparatus and monitoring the resulting concentration at consecutive stages.

The odor stream exiting from the diluter at each stage was analyzed with a flame ionization detector (FID) hydrocarbon analyzer. The FID was calibrated to give carbon concentration in ppm, which is also the concentration of methane.

Starting with the most dilute output concentration, mixer rotameters were adjusted while stepping through each stage of dilution. Adjustment was made such that the methane concentration detected at each stage increased threefold over the previous stage. At each stage rotameter readings were recorded for reference. Once a potential set of readings had been attained, the dilution was repeated twice to validate

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the proper rotameter setting for each desired dilution. The concentrations varied less than 10 percent, which was acceptable.

The odor containing gas stream produced by the diluter was plumbed to a manifold located inside the test chamber. Teflon tubing connected to a tee in the manifold established a flow path to the inside of a M17 respirator. The respirator had been modified by installation of bulkhead fittings which penetrated the charcoal filter cartridges of the mask. In this way, the organic vapor could reach the mask interior without being attenuated by the charcoal, and would be conditioned by the temperature, humidity, and interior surface of the mask in much the same way as in-leakage would be. The gas flow rate through the manifold was adequate to supply all the breathing requirements of the mask wearer. The large bore (1.3 cm) tubing connecting the mask to the manifold permitted the subject to inhale without the burden of a high pressure drop, or the insult of a high gas flow rate. The air flow rate was measured using the rotameters in the air supply and diluter described above. The temperature and relative humidity of the air stream were measured with a type K thermocouple and a dewpoint hygrometer (EG&G Model 911).

The subject's exhaled air exits the mask through the exhalation valve, as usual, and is carried away in the clean air flow which continuously purges the chamber. This purge flow enters the chamber at the ceiling and exits to an exhaust hood through plumbing connected to the chamber floor.

Samples of the odor containing gas stream were collected at test conditions for determination of the delivered concentrations. These samples were collected by directing a measured flow of the gas through a solid sorbent sampling device. The mass of the odorant compound collected was determined by desorbing the sampler into a gas chromatograph (GC) equipped with a flame ionization detector (FID). The GC was calibrated by injections of known volumes of each of the compounds to

generate a response curve. The low mass concentrations of nonanoic acid and skatole used in this testing were below the detection limit of the GC system. For these two compounds, calculated concentrations were based upon empirically determined vapor pressures and measured test temperatures and flow rates.

## 3.2.3 Data Reduction Method

Primarily, data analysis consisted of determination of odor threshold values. As discussed above, several odor threshold values may be defined. For our purpose, threshold values reported are for those of detection of an odor's presence and not for the ability to recognize the odor as the one which is being studied. When we refer below to an odor threshold value it is this detection threshold, not a recognition threshold value. The detection threshold is typically lower than a recognition threshold for an odor. The difference between the two threshold concentrations depends upon the odor, and is not predictable with much certainty.

The procedure used to determine the odor threshold of a compound, based on the panel's response, has been described elsewhere[17,18] but will be summarized here. The lowest concentration at which a subject correctly identified the odorant stream followed by two additional correct responses at successive higher concentrations was used as the basis for calculating an individual's threshold. The value of a subject's threshold was then computed as the geometric mean of the lowest concentration identified and the next lower concentration used in the series. The 100 percent odor detection threshold for the panel is, of course, the highest recorded threshold for any member. The 50 percent odor detection threshold represents the median value of the panel's individual thresholds.

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## 3.3    Odor Threshold Testing Results.

In Table 5 is a summary of the results from the odor threshold testing, presented in terms of the number of subjects which made their first correct response of three consecutive correct responses at each stage. For example, considering tBM, six of the 19 subjects in the total group had their first of three consecutive correct responses to identifying which stream contained in the odorant at stage 6. The table shows the frequency for the collective sample population as well as the four classes--whites, blacks, smokers, and nonsmokers. Keep in mind that comparisons cannot be made between the compounds in this table because the concentrations were different for each at a given stage of dilution.

Several subjects correctly identified tBM, nonanoic acid, and skatole at stage 8, but most of these subjects strictly guessed correctly at stage 8 and could not detect an odor with certainty until a later stage. The statement is corroborated by subject comments after testing in which they stated guesses were generally made in stage 8; although, a few isolated cases existed where a subject could detect an odor at stage 8. Another point that can be made is that there is a distribution of the concentrations at which each odorant could be detected. The tBM and nonanoic acid had the most consistent results in that nearly all the subjects could detect the odorant within a three-stage region. The remaining three compounds show more spread in the distribution, especially with IAA. Eventually, all the subjects were able to detect the odorant stream for each compound.

Table 6 contains a summary of the experimental and calculated odorant concentrations of each compound for every stage of dilution. The experimental values have been determined by GC analysis and the calculated values result from theoretical calculations based on flow rates and vapor pressures. For IAA and MeS there is reasonably close agreement between calculated and experimental concentrations. On the other hand, the agreement between experimental and calculated values for tBM is poor.

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TABLE 5.   FREQUENCY OF A SUBJECT'S FIRST OF THREE CORRECT
RESPONSES AT EACH STAGE OF DILUTION FOR EACH
SAMPLE CLASS AND FOR EVERY CANDIDATE COMPOUND

| Compound | Sample Population | Dilution Stage | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 8 | 7 | 6 | 5 | 4 | 3 | 2 |
| tBM | Total | 5 | 4 | 6 | 0 | 1 | 3 | 0 |
| | Whites | 4 | 3 | 5 | 0 | 1 | 3 | 0 |
| | Blacks | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| | Smokers | 3 | 2 | 2 | 0 | 0 | 1 | 0 |
| | Nonsmokers | 2 | 2 | 4 | 0 | 1 | 2 | 0 |
| IAA | Total | 2 | 5 | 5 | 2 | 4 | 1 | 0 |
| | Whites | 2 | 3 | 5 | 1 | 4 | 1 | 0 |
| | Blacks | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| | Smokers | 1 | 1 | 2 | 0 | 3 | 1 | 0 |
| | Nonsmokers | 1 | 4 | 3 | 2 | 1 | 0 | 0 |
| MeS | Total | 1 | 8 | 3 | 2 | 1 | 4 | 0 |
| | Whites | 1 | 7 | 2 | 2 | 1 | 3 | 0 |
| | Blacks | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| | Smokers | 0 | 2 | 2 | 1 | 1 | 2 | 0 |
| | Nonsmokers | 1 | 6 | 1 | 1 | 0 | 2 | 0 |
| Nonanoic acid | Total | 3 | 5 | 7 | 1 | 1 | 2 | 0 |
| | Whites | 3 | 3 | 6 | 1 | 1 | 2 | 0 |
| | Blacks | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| | Smokers | 1 | 1 | 3 | 1 | 1 | 1 | 0 |
| | Nonsmokers | 2 | 4 | 4 | 0 | 0 | 1 | 0 |
| Skatole | Total | 4 | 2 | 7 | 3 | 3 | 0 | 0 |
| | Whites | 3 | 1 | 7 | 2 | 3 | 0 | 0 |
| | Blacks | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| | Smokers | 0 | 2 | 3 | 2 | 1 | 0 | 0 |
| | Nonsmokers | 4 | 0 | 4 | 1 | 2 | 0 | 0 |

TABLE 6.  EXPERIMENTAL AND CALCULATED CONCENTRATION OF CANDIDATE
COMPOUNDS AT EACH STAGE OF DILUTION (ng/l)

| Compound | | Dilution Stage | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 8 | 7 | 6 | 5 | 4 | 3 | 2 |
| tBM | Exp | 0.0028 | 0.0085 | 0.026 | 0.077 | 0.23 | 0.69 | 2.1 |
| | Calc | 0.00048 | 0.0014 | 0.0043 | 0.013 | 0.039 | 0.12 | 0.35 |
| IAA | Exp | 2.9 | 8.6 | 26 | 78 | 230 | 700 | 2100 |
| | Calc | 4.8 | 15 | 44 | 130 | 390 | 1200 | 3500 |
| MeS | Exp | 0.11 | 0.34 | 1.0 | 3.1 | 9.2 | 27 | 83 |
| | Calc | 0.32 | 0.95 | 2.9 | 8.6 | 26 | 77 | 230 |
| Nonanoic acid | Exp[a] | -- | -- | -- | -- | -- | -- | -- |
| | Calc | 0.39 | 1.2 | 3.5 | 11 | 32 | 95 | 290 |
| Skatole | Exp[a] | -- | -- | -- | -- | -- | -- | -- |
| | Calc | 1.9 E-5 | 5.8 E-5 | 1.7 E-4 | 5.2 E-4 | 1.6 E-3 | 4.7 E-3 | 1.4 E-2 |

(a) Experimental values were not determined due to detection limits of the GC.

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The poor agreement is likely due to the fact that tBM was in solution with water. The calculation was made assuming Henry's law with a constant coefficient. It's apparent that the assumptions made did not hold. The experimental value is considered to be the correct value. For nonanoic acid and skatole, experimental concentrations were not determined because the GC was unable to be calibrated for nonanoic acid and the GC was not sensitive enough to detect the trace amounts of skatole that could be collected.

Table 7 presents the 50 percent odor threshold concentration determined from the experiment for each of the candidate compounds and compares them to published values[1] of the 50 percent recognition threshold. The odor threshold concentration has been calculated as the sample median of the individual odor thresholds. The lower thresholds measured here are consistent with the fact that these values are for detection and published thresholds are for the 50 percentile recognition concentrations. For tBM, more than an order of magnitude separates the experimental and published threshold concentrations, which is a much larger difference than any of the other four compounds.

Given in Table 8 is a summary of the median and 100 percent odor detection thresholds measured for every compound and for each group within the sample population. No significant comparison can be made between the race groups since 16 of the 19 subjects tested were white. With nearly equal number of smokers and nonsmokers, however, comparison between these groups can be made. For three of the five compounds, nonsmokers appeared to be more sensitive to the candidate odorant. The sensitivity does not seem to be pronounced, however, in that the 50 and/or 100 percent detection thresholds were different only by about a factor of three (one dilution stage). Conversely, smokers appear to be somewhat more sensitive to the mercaptan than nonsmokers. No reason can be given for this phenomenon. Finally, there was no difference between smokers and nonsmokers for skatole.

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TABLE 7. ODOR THRESHOLDS (CALCULATED, EXPERIMENTAL, AND
PUBLISHED VALUES) FOR THE FIVE COMPOUNDS FOR
THE SAMPLE POPULATION

| Compound | Odor Threshold Concentrations, ng/l | | |
|---|---|---|---|
| | Calculated | Experimental | Published[1] |
| tBM | 0.0025 | 0.015 | 0.3 |
| IAA | 25 | 15 | 81 |
| MeS | 1.7 | 0.59 | 3.7 |
| Nonanoic acid | 2.0 | --[a] | 5.5 |
| Skatole | 1.0 E-4 | --[a] | 4.0 E-4 |

(a) Experimental values not obtained because of detection limits of the GC.

TABLE 8. DETECTION THRESHOLD CONCENTRATIONS (ng/1)

| Sample | tBM | | IAA | | MeS | | Nonanoic Acid | | Skatole | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 50% | 100% | 50% | 100% | 50% | 100% | 50% | 100% | 50% | 100% |
| Collective | 0.015 | 0.40 | 15 | 400 | 0.59 | 16 | 2.0 | 55 | 1.0 E-4 | 9.1 E-4 |
| Whites | 0.015 | 0.40 | 15 | 400 | 0.34 | 16 | 2.0 | 55 | 1.0 E-4 | 9.1 E-4 |
| Blacks | 0.0049 | 0.015 | 5.0 | 45 | 0.59 | 16 | 0.68 | 2.0 | 3.4 E-5 | 3.0 E-4 |
| Smokers | 0.0049 | 0.40 | 45 | 400 | 1.0 | 16 | 2.0 | 55 | 1.0 E-4 | 9.1 E-4 |
| Nonsmokers | 0.015 | 0.40 | 15 | 130 | 0.20 | 16 | 0.68 | 55 | 1.0 E-4 | 9.1 E-4 |

## 3.4    Conclusions Regarding Odor Threshold Results.

Examination of the results presented in Tables 7 and 8 clearly indicate large differences between the thresholds for the test compounds, with IAA being the most difficult one to detect. Our results follow the same ranking as previously published results indicate for these compounds, although the values measured here are somewhat lower, in general. There are no differences between the threshold values for the groups of subjects listed in Table 8 which would cause any of the compounds to be rejected from further consideration.

The data in Table 7 can be combined with the vapor pressure for the candidates to calculate the odor index for each compound. The odor index is simply the compound's equilibrium vapor concentration in air at a specified temperature, divided by the odor threshold concentration. The larger the value of this parameter, the higher the protection factor which could be detected by a masked subject exposed to a challenge of the compound.

Using this criterion, IAA, with an odor index of $2.8 \times 10^6$ should outperform MeS and nonanoic acid, which have values of $7.1 \times 10^5$ and $4.5 \times 10^5$. Despite the fact that the odor threshold concentration of IAA is higher than that of those two compounds, its higher vapor pressure more than compensates for the need for higher concentrations.

The remaining two candidate compounds have odor indices much greater than that of IAA. Skatole has a very low odor threshold, but also has a very low vapor pressure at ambient temperatures. It should be noted that the vapor pressure at 20°C we have calculated (0.038 mmHg) is based on an extrapolation of data[1] which are for the temperature range 95°C < T, and is therefore uncertain. Using this calculated figure, the odor index for skatole is $2.7 \times 10^9$. The high vapor pressure of tBM results in an even higher odor index for this compound, $5.0 \times 10^{10}$. Based upon these figures, tBM and skatole were selected for inclusion

with IAA in the qualitative versus quantitative testing performed in the next phase of this project.

4.        QUALITATIVE/QUANTITATIVE FIT TESTING

The final phase of this investigation was the performance of simultaneous qualitative and quantitative fit testing using the odorants identified as having the best potential for use during the odor threshold testing described above.  The objective of this set of experiments was to measure the performance of tBM and skatole under simulated fit testing conditions and to compare their performance with that of IAA.

4.1       Procedure for Combined Qualitative
          and Quantitative Fit Testing.

The procedure used in these tests was designed to place the subject in a constant challenge of odorant and corn oil aerosol, while he created increasing amounts of leakage into the modified mask.  The mask protection factor is measured by means of the corn oil aerosol concentration inside the mask at each level of leakage until the subject clearly detects the odorant.

The odorant challenge concentrations were initially selected to be 10,000 times the measured odor threshold concentration reported above. By setting the challenge concentration for each odorant at a fixed multiple of its threshold concentration, the same experimental apparatus and protocol could be used during the qualitative/quantitative testing to achieve comparable results.  In this way, if experimental conditions were held perfectly constant for all tests and there were no subject-to-subject variations in odor sensitivity, each odorant would be detected at the same PF in all cases.  If, instead of scaling the odorant challenge concentration according to its odor threshold, a common challenge concentration was used for all three odorants, great variations in the amount of mask leakage would be needed.  If, for example, tBM were

detected at PF = 1000, skatole would be detected at PF = 125000, and IAA would not be detected until PF = 1.

Before testing began, trial runs with a few subjects were performed to verify that the odor could be detected at an intermediate leakage in the test protocol. The target concentrations for the three odorants during this testing were: 250 mg/m$^3$ for IAA, 250 $\mu$g/m$^3$ for tBM, and 2 $\mu$g/m$^3$ for skatole. It was not the intention here to produce the concentrations of odorant one might anticipate in field fit testing because that is a variable quantity depending upon environmental conditions and the method of test administration. The concentrations of IAA and tBM achieved in the tests were measured by collection of a gas-tight syringe sample of the test chamber atmosphere during testing, followed by analysis by GC-FID. The skatole concentrations used proved to be below the detection limit of the analytical system used, so the value calculated from the generator operating conditions is reported.

The method of challenge odorant generation varied with the compound used. The IAA challenge was generated by passing a controlled flow of filtered air through a bubbler containing pure IAA liquid at a constant temperature. The skatole challenge was generated similarly, except that a saturated aqueous solution of skatole was used in the bubbler. The tBM challenge was generated by injecting a steady flow of tBM in a nitrogen pressurized cylinder into the delivered gas stream. In each case, the odorant containing gas stream was greatly diluted immediately after exiting the vapor generation device.

The measurements performed during these tests include the odorant gas flow rates, aerosol generator flow rate, and the total gas flow rate into the test chamber. The challenge odorant concentrations were measured as indicated above. The corn oil aerosol was measured in samples of air taken from inside the chamber and from inside the mask worn by the test subjects. The aerosol measurements were performed using a Laser Aerosol Spectrometer (LAS-X) manufactured by Particle Measurement

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Systems (Boulder CO). This instrument samples the air at a constant rate of 90 cc/min, and passes the air sample through a light scattering chamber where individual particles scatter light from a He-Ne laser beam. Particles ranging in size from 0.1 to 3.0 $\mu$m are sized and their count is accumulated in discrete size bins for further processing. From the measured size distributions, the mass concentrations of aerosol inside and outside the mask are calculated. The mass concentration outside the mask is divided by that inside the mask to yield the protection factor.

The subjects donned a modified M17 respirator for these tests. The quality of the fit was checked by performing a negative pressure check before they entered the test chamber. A septum was installed in each of the lenses of the M17, and each septum contained capillaries which were initially sealed. A sampling port was attached to the mask on the centerline, just above the lenses of the mask. The subject entered the chamber after the aerosol and odorant concentrations were established and connected the mask sampling line to the appropriate port on the chamber bulkhead. The initial protection factor was determined by measuring the aerosol concentration in the challenge atmosphere and inside the respirator. The subject then proceeded to open the capillaries in order of increasing diameter, permitting the aerosol concentration inside the mask to be measured after each capillary was opened. The capillary sizes used were: 27, 25, 23 (x2), and 22 ga. The subject was seated and instructed to remain still throughout the test to avoid generating spurious leaks. The subject indicated after opening each capillary whether he detected the odor or not. After the odor was detected at two consecutive capillaries and the aerosol concentrations inside the mask were measured, the challenge aerosol concentration was remeasured. The subject then disconnected the sampling line and exited the chamber.

One final point to be made regarding the test procedure is that the subject pool varied for the three odorant compounds. Ideally, the same subjects would be used for all three of the odorants examined, but

the volunteers' schedules made their participation impossible in some cases, and several of the subjects refused to participate in the tests involving skatole. Despite the variation in the subject pool composition, the data obtained permit clear conclusions to be drawn regarding the potential utility of the candidate compounds studied.

4.2     Results of Qualitative/Quantitative
        Respirator Fit Testing.

The results obtained using each of the odorants, IAA, tBM, and skatole are discussed separately in the following paragraphs. One must keep in mind that different challenge concentrations were used for the different compounds. By changing the challenge odorant concentration, one can change the value of the PF at which it is detected by the subject. Comparisons between the results presented here must take that fact into account.

4.2.1     Results for Isoamyl Acetate (IAA).

A total of 16 subjects were tested as described above using the odorant isoamyl acetate (IAA). One of the subjects (GW) was tested twice because he failed to detect the odor of IAA at any point during the initial test. The PFs measured at each step in the test for all subjects, and the PF at the point the odorant was detected are shown in Table 9.

The PFs obtained by the subjects prior to initiating any of the capillary leaks range from 36,000 to 230,000. One must keep in mind that all measurements of PF were performed with the subject remaining motionless and rigorously avoiding any facial expressions. This accounts for the high initial values of PF. The measured PF values decrease as each successive capillary is opened to permit higher leakage rates into the mask. The PF at which the subjects detected the odor of IAA range from 470 to 9000, if we discount the one trial in which the subject never

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TABLE 9. PROTECTION FACTORS ($\times 10^3$) MEASURED FOR SUBJECTS
DURING IAA QUALITATIVE-QUANTITATIVE FIT TEST
(Underlined Value Denotes PF at Odor Detection
by Subject)

| Subject ID | Capillaries Opened | | | | | |
|---|---|---|---|---|---|---|
| | None | 27 ga | 25 ga | 23 ga | 23 ga | 22 ga |
| KCH | 140 | 7.3 | 5.0 | 2.6 | _1.6_ | 1.5 |
| JJD | 36 | 6.4 | 4.3 | 0.63 | _0.61_ | 0.31 |
| JMB | 100 | 5.1 | 2.0 | 0.91 | 0.72 | _0.47_ |
| TJK | 200 | 28 | 22 | _1.0_ | -- | -- |
| NKR | 90 | 6.2 | 2.5 | 2.7 | _1.1_ | 0.66 |
| RAS | 120 | 5.7 | _2.1_ | 2.2 | 1.3 | -- |
| SLB | 87 | 82 | 9.7 | _9.0_ | 0.69 | -- |
| GDN | 94 | 18 | 5.4 | (a) | (a) | _0.62_ |
| CWM | 97 | 3.4 | 2.4 | _2.1_ | 1.0 | -- |
| MDA | 160 | 13 | 3.1 | 5.5 | -- | _1.2_ |
| SAF | 150 | 5.4 | 4.9 | 4.7 | -- | _1.1_ |
| GW1 (b) | 140 | 4.0 | 1.8 | 1.8 | -- | 0.85 |
| MRK | 61 | 45 | 16 | _2.8_ | 1.7 | -- |
| GW2 | 230 | 12 | 1.7 | 1.4 | _1.5_ | 0.31 |
| TCZ | 89 | 14 | _4.4_ | 0.82 | -- | -- |
| DOM | 58 | 11 | 2.0 | 2.1 | 1.5 | _0.90_ |
| GMT | 120 | 4.7 | 4.1 | _3.4_ | 3.2 | -- |

(a) Subject removed capillary from mask system.
(b) Subject never detected odor.

detected the odor. This span of over one order of magnitude is consistent with the measured range of the odor threshold concentration for a subject pool of this size.

The IAA challenge concentrations varied from 200 to 270 mg/m$^3$ for these tests except for two subjects which fell outside that range. Subject JJD's challenge concentration was 160 mg/m$^3$, and that for MRK was 330 mg/m$^3$. The concentration of IAA inside the mask is equal to the challenge concentration divided by the measured protection factor, if one assumes the challenge aerosol and vapor leak into the mask equally. The range of concentrations at which the subjects detected IAA was 0.03 to 0.54 mg/m$^3$, with an average value of 0.20 mg/m$^3$. This can be compared against our measured odor threshold value of 0.015 mg/m$^3$ reported in a previous section of this report. A possible cause for this discrepancy may lie in the manner of odor presentation to the subjects. A principal difference between the odor threshold testing and the qualitative/quantitative fit testing is the change in odor concentration which occurs at the time the subject must identify the odor. In odor threshold testing, the subject switches from a clean air stream to one containing the odorant at a given concentration. This produces a sudden change in the odor concentration experienced, which is large, compared to the changes in concentration experienced when consecutive capillaries are opened in the mask. It is easier for the subject to detect the odor when the rate of change of concentration is greater, so the reported threshold concentrations are lower than the values implied by the quantitative fit testing. Thus, if a subject wearing a leaky mask suddenly steps from a clean atmosphere into one containing the odorant at the challenge concentration used here, he would be likely to detect it at a somewhat higher PF than Tables 9 through 11 indicate. This effect should be observed to about the same degree for any odorant, so it will not affect the conclusions we can draw from these results.

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TABLE 10.  PROTECTION FACTORS (x10$^3$) MEASURED FOR SUBJECTS
DURING tBM QUALITATIVE-QUANTITATIVE FIT TEST
(Underlined Value Denotes PF at Odor Detection
by Subject)

| Subject ID | Capillaries Opened | | | | | |
|---|---|---|---|---|---|---|
| | None | 27 ga | 25 ga | 23 ga | 23 ga | 22 ga |
| SAF | 120 | 150 | 27 | 2.8 | 1.9 | <u>0.4</u> |
| CB | 36 | 79 | <u>20</u> | 12 | -- | -- |
| MDA | 23 | 19 | 6.2 | 3.8 | 1.2 | <u>0.3</u> |
| GMT | 130 | 140 | <u>12</u> | 3.7 | -- | -- |
| GRW | 77 | 28 | <u>3.1</u> | 3.1 | -- | -- |
| NKR | 21 | 34 | <u>4.3</u> | 1.8 | -- | -- |
| CWM | 24 | 26 | 4.3 | 2.8 | 4.0 | <u>1.2</u> |
| GDN | 40 | 8.8 | 9.5 | 6.3 | 6.0 | <u>0.6</u> |
| JMB | 26 | 4.6 | 2.1 | 1.4 | <u>0.8</u> | 0.3 |
| TJK | 35 | <u>19</u> | 18 | 22 | -- | -- |
| PG | 45 | 8.0 | 3.2 | (a) | 3.5 | <u>1.9</u> |
| KCH | 7.0 | 3.8 | 1.9 | 2.0 | <u>1.5</u> | 0.3 |
| JJD | 22 | 14 | 6.7 | <u>5.6</u> | 1.4 | -- |
| MRK | 12 | 10 | 11 | <u>1.6</u> | 0.8 | -- |
| DOM | 18 | 9.6 | 4.8 | <u>0.8</u> | 0.6 | -- |
| RAS | 22 | (a) | <u>1.9</u> | 0.8 | -- | -- |

(a)  Subject removed capillary from septum without opening it.

TABLE 11. PROTECTION FACTORS ($\times 10^3$) MEASURED FOR SUBJECTS
DURING SKATOLE QUALITATIVE-QUANTITATIVE FIT TEST
(Underlined Value Denotes PF at Odor Detection
by Subject)

| Subject ID | Capillaries Opened | | | | | |
|---|---|---|---|---|---|---|
| | None | 27 ga | 25 ga | 23 ga | 23 ga | 22 ga |
| GMT | 180 | 210 | 12 | (a) | 3.5 | <u>1.5</u> |
| GDN[b] | 170 | 25 | 5.0 | 1.2 | 0.7 | 0.3 |
| AJK | 230 | 180 | 6.9 | 3.2 | <u>3.0</u> | 1.1 |
| KCH | 120 | 250 | 5.6 | 2.2 | 2.2 | <u>0.7</u> |
| MRK | 23 | 4.3 | 5.7 | <u>2.6</u> | 2.3 | -- |
| JMB | 99 | 7.5 | 2.5 | 1.3 | <u>0.9</u> | 0.4 |
| SRB | 310 | 110 | 8.0 | <u>1.3</u> | 0.7 | -- |
| SLB | 74 | 20 | 5.7 | 0.9 | <u>0.7</u> | 0.4 |
| TJK | 170 | 100 | <u>13</u> | 22 | 4.2 | 1.0 |
| MDA | 130 | 60 | 5.7 | 2.1 | 1.3 | <u>0.7</u> |

(a) Subject opened both 23 ga capillaries simultaneously.
(b) Subject never detected odor.

The data presented in Table 9 demonstrate that, in all but one instance, the subjects eventually detected the odor of IAA, while none of them detected it until at least two capillaries had been opened. This confirmed that the vapor challenge concentration was an appropriate value. Figure 1 presents these data in a graphical form, illustrating the range of PFs measured for all subjects as each successive capillary is opened. Two points can be made with the aid of this graph. First, the range of PF values obtained for any given leakage area decreases as the leakage area increases. This occurs because the small leakage around the face mask periphery, which varies greatly for the subjects, is eventually dominated by the leakage induced through the open capillaries. That the initial leakage through the mask periphery is quite small can be surmised from the obvious drop in PF seen in Figure 1 when a controlled leakage area of 0.003 cm$^2$ is opened. Second, the PF values at which the subjects detected the odor do not display any pattern with respect to the leakage area. This is an indication that the odor detection is not an experimental artifact. These PFs are, in fact, approximately normally distributed about their median value of 1350.

## 4.2.2    Results for t-Butyl Mercaptan (tBM).

Table 10 contains the data obtained using this candidate compound. These results are qualitatively similar to those obtained for IAA. There are, however, several instances in these data where the measured PF increased when additional leakage area was opened. See, for example, subject SAF, whose PF increased from 120,000 to 150,000 after he opened the 27 gauge capillary. This phenomenon is believed to be caused by variations in subject breathing patterns or by inadvertent facial movements by the subjects. The period of time through which the subjects were sampled at each step was not long enough to eliminate the influence of such subject actions in every case.
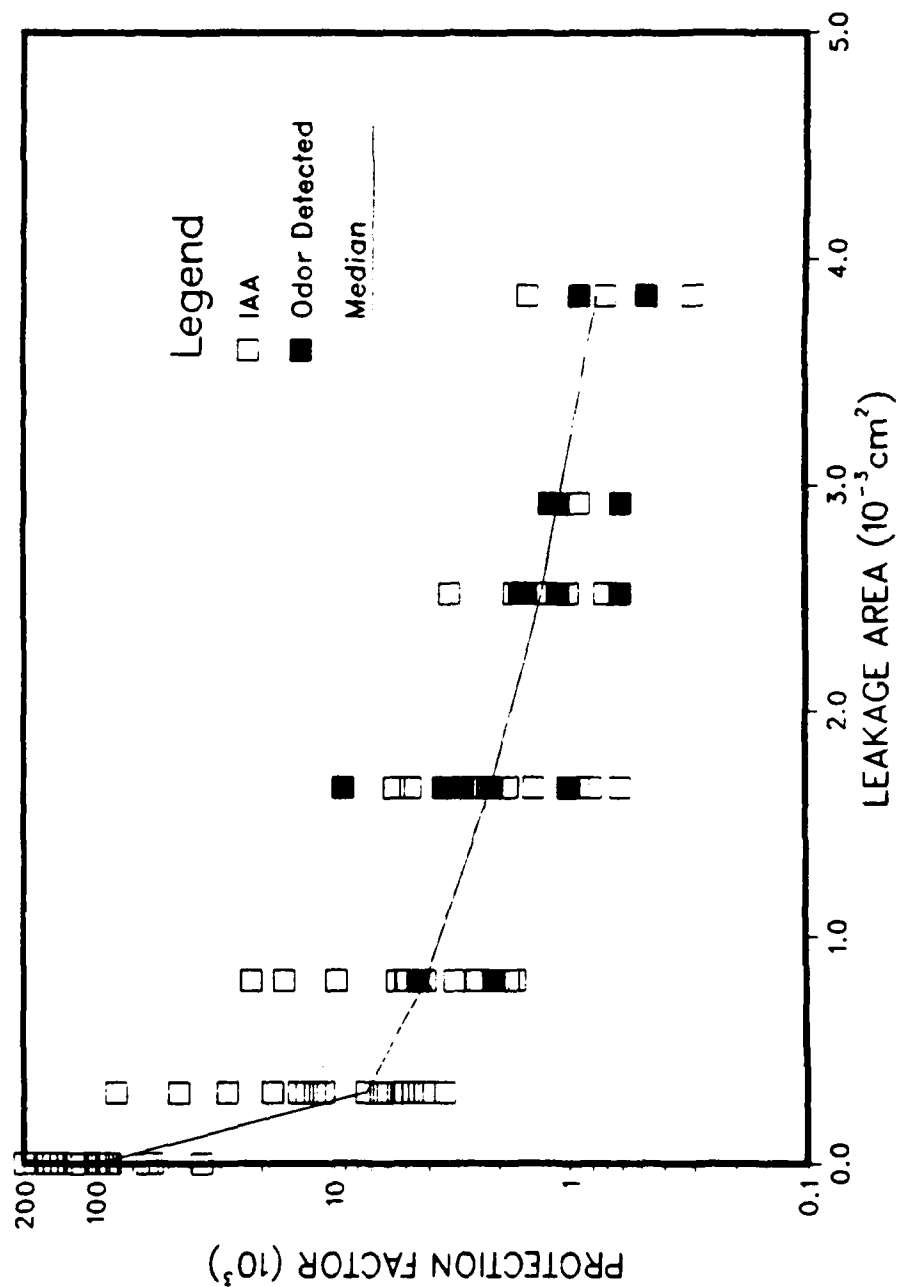
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FIGURE 1. PROTECTION FACTORS FOR IAA TESTING OF ALL SUBJECTS AS A FUNCTION OF LEAKAGE AREA OF THE OPEN CAPILLARIES. (Solid points indicate data corresponding to subjects' detection of the odor.)

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The vapor challenge concentration used in the tBM testing was more easily reproduced than was that of IAA, because liquid levels and temperatures of the vapor generator were not involved. The tBM flow rate from the gas cylinder was monitored during testing to ensure a uniform challenge concentration. The syringe samples collected from the chamber indicated an average concentration of 236 $\mu g/m^3$, with a standard deviation of only 12 $\mu g/m^3$.

This challenge concentration, and a mean PF at detection of 4700 implies a threshold concentration of tBM inside the respirator of 0.05 $\mu g/m^3$. This is a bit more than three times the figure (0.015 $\mu g/m^3$) determined from the odor threshold testing reported above.

Figure 2 depicts the same data as does Table 10. As was seen to be the case with IAA, the spread in protection factors measured at each stage of the testing is approximately an order of magnitude, decreasing with increasing leakage area. Again, the odor was detected by the subjects throughout the range of open capillary areas, and the PFs at which this occurred are distributed evenly about the median value of 1750.

4.2.3    Results for Skatole.

The data obtained in the qualitative-quantitative fit testing performed with the odorant skatole are presented in Table 11 and in Figure 3. The number of volunteers to participate in this phase of the testing decreased from the previous pool because of the disagreeable odor of this compound. Although the low concentration experienced inside the mask does not produce, for most subjects, an unpleasant sensation, the challenge concentration is another matter. The vapor of this compound also adsorbs to most surfaces because of its very low vapor pressure and produces a detectable odor for a prolonged period of time. This feature of the compound makes it difficult to envision its effective use in a field testing situation.

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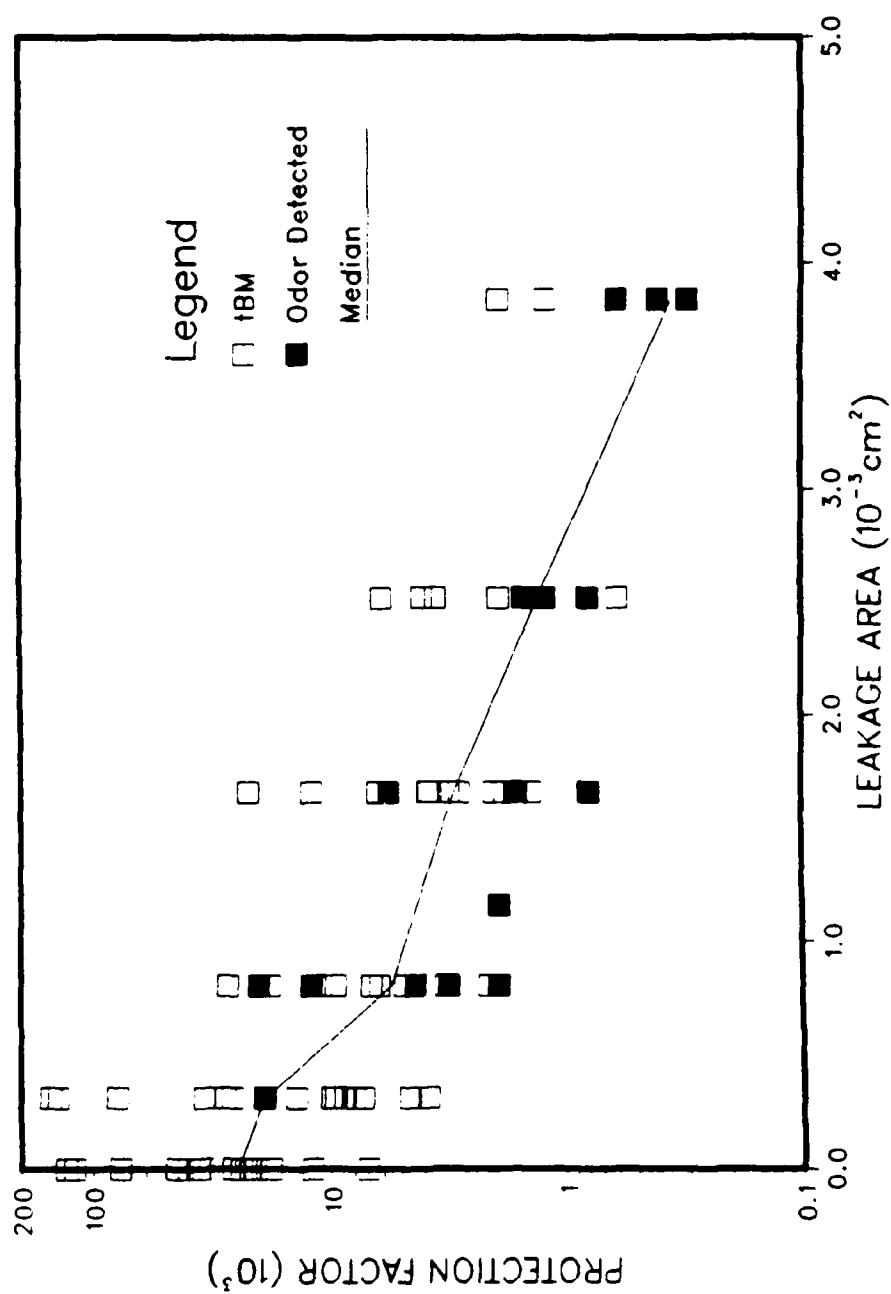FIGURE 2. PROTECTION FACTORS FOR tBM TESTING OF ALL SUBJECTS AS A FUNCTION OF LEAKAGE AREA OF THE OPEN CAPILLARIES. (Solid points indicate data corresponding to subjects' detection of the odor.)
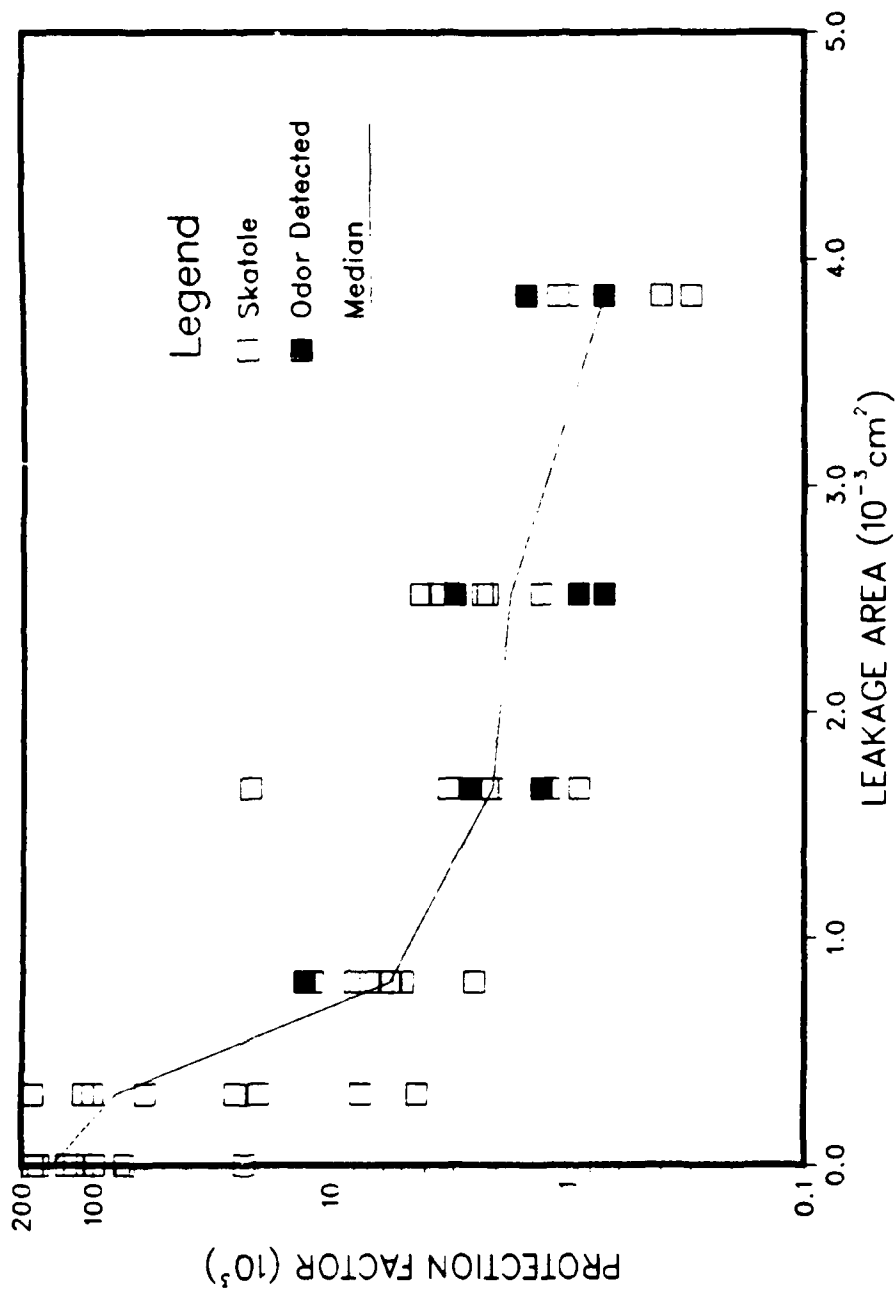
FIGURE 3.  PROTECTION FACTORS FOR SKATOLE TESTING OF ALL SUBJECTS AS A FUNCTION OF LEAKAGE AREA OF THE OPEN CAPILLARIES.  (Solid points indicate data corresponding to subjects' detection of the odor.)

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The general trends seen in Figures 1 and 2 are present again in the data shown in Figure 3. The median PF at which the odor was detected is 1300, and the set of values for the subjects are evenly distributed about this value.

The target challenge concentration for skatole was 2 $\mu g/m^3$ and was generated using a bubbler technique as described above. This concentration, while eminently detectable by its odor, is beneath the sensitivity of the GC-FID system used to characterize the odor challenge levels. If one assumes that the target concentration was achieved, the in-mask average odor detection threshold measured here is about 1.5 $ng/m^3$ or 15 times the value measured in the odor threshold testing. This disparity is very similar to that found for IAA.

### 4.2.4   Summary of Results of Qualitative-Quantitative Fit Testing.

Summary statistics are given in Table 12 regarding the PFs at which the three odorant compounds were detected by the subjects in the testing. It is reemphasized that one can change the value of PF at which the odorant is detected by changing the challenge gas concentration. The challenge concentrations selected for use here differ by more than four orders of magnitude and were intended to enable detection of the three compounds at similar PF values. The mean values given in Table 12 indicate that the concentrations used were appropriate. For each of the compounds, the spread in the distribution, as indicated by the standard deviation, is as large or larger than the mean value. The use of a much larger subject pool would be expected to reduce the relative standard deviations only slightly. Because of the variability in subjects' sensitivity to odor, we anticipate that results very similar to these would be achieved for any odorous vapor used at an appropriate challenge concentration.

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TABLE 12. SUMMARY STATISTICS OF THE DISTRIBUTION OF PFS AT
WHICH THE THREE ODORANTS WERE DETECTED

| Compound | Mean Challenge Concentration | | $\overline{PF}$ | Standard Deviation | $PF_{max}$ | $PF_{min}$ | n(a) |
|----------|------------|-------|-----|-----------|--------|--------|------|
|          | $\mu g/m^3$ | ppm | | | | | |
| IAA | 211,000 | 39 | 2100 | 2100 | 9000 | 470 | 16 |
| tBM | 236 | 0.043 | 4700 | 6500 | 20000 | 300 | 16 |
| Skatole | 2(b) | 0.0005 | 2700 | 4000 | 13000 | 700 | 9 |

(a) Number of subjects. Those not detecting the compound are omitted from these statistics.

(b) This is a calculated value, based upon operating conditions.

The median values of the PF measured for all subjects at the design values of leakage area are presented in Figure 4 for the three compounds. This is not meant to imply that the PF is a function of the odorant used. Rather, it is an indication of the degree of reproducibility which was achieved in these experiments. The much greater scatter in these curves for leakage areas less than 0.005 $cm^2$ is a result of the fact that the quality of the face seal achieved is variable and dominates the initial leakage. As the total amount of open capillary area increases, the controlled leakage dominates and results in more reproducible PFs for a pool of subjects. Clearly, there is still subject to subject variability in the PF at each stage of leakage, but, as was pointed out before, this decreases with increasing controlled leakage area.

5.      CONCLUSIONS AND RECOMMENDATIONS

The work reported here has resulted in the identification of a group of safe compounds which have potential for use as qualitative fit test simulants. For a selected subset of those compounds, odor thresholds were successfully determined using a panel of subjects. The results obtained in that portion of the testing identified two of the four candidates as being likely to outperform isoamyl acetate as an odorant challenge in qualitative fit testing.

Simultaneous qualitative/quantitative respirator fit testing was performed successfully for pools of subjects using isoamyl acetate, t-butyl mercaptan, and skatole. The results obtained in that testing permit us to draw the following conclusions:

  • t-Butyl mercaptan has a much higher ratio of
    vapor pressure to odor threshold than does
    isoamyl acetate and meets all the selection
    criteria identified at the outset of the
    project. The concentration of t-butyl mercaptan
    used in this testing was less than 250 $\mu g/m^3$,
    and permitted an average PF detection of 4700.
    These figures are to be compared against a

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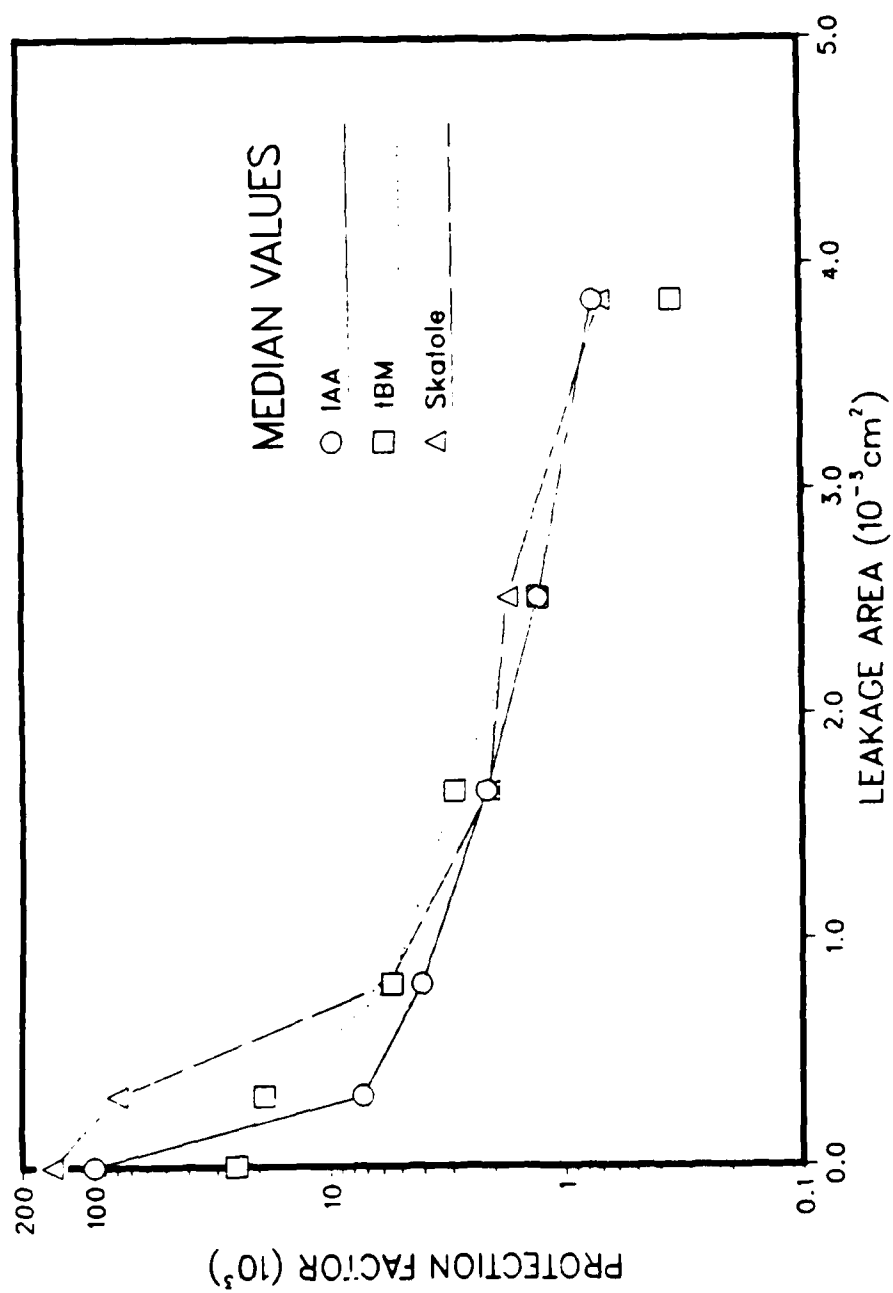
FIGURE 4. MEDIAN VALUES OF PFs MEASURED AT DESIGN LEAKAGE AREA VALUES FOR ALL SUBJECTS USING THE THREE ODORANT COMPOUNDS

challenge concentration of about 250 mg/m$^3$ for
isoamyl acetate required for detection of a PF
of 2100. Increasing the challenge concentration
of t-butyl mercaptan can significantly increase
the PF values which can be determined in a
qualitative test.

- Although skatole has a lower odor threshold
  concentration than most compounds examined, it
  is not especially well suited for use in
  qualitative fit testing. At very low concen-
  trations, such as are found in the tested mask,
  the compound is not particularly malodorous.
  The challenge vapor, because of its relatively
  low vapor pressure, deposits on surfaces such
  as the subject's clothing. In a field setting
  this would result in contamination of the test
  area by the odor to be detected and would be
  likely to adversely affect the testing.

Based on the results obtained in this work, we recommend that
t-butyl mercaptan be investigated further as a supplemental or replace-
ment qualitative fit test simulant. We believe it is appropriate at this
time to conduct limited simultaneous qualitative/quantitative respirator
fit testing on troops in the field. The use of dilute solutions of
t-butyl mercaptan should be examined for generation of challenge
atmospheres suitable for qualitative detection of protection factors on
the order of 10,000.

Blank

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# APPENDIX A


## CANDIDATES EXAMINED AS POTENTIAL QUALITATIVE FIT TEST SIMULANTS

## TABLE A-1. CANDIDATES EXAMINED AS POTENTIAL QUALITATIVE FIT TEST SIMULANTS

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Acetaldehyde | 0.3 | 4,300,000 | B |
| Acetic acid | 1.0 | 15,000 | A |
| Acetic anhydride | 0.36 | 14,611 | A |
| Acetone | 140 | 720 | A |
| Acetonitrile | 35[b] | 2,400 | A |
| Acetophenone | 0.6 | 2,183 | A |
| Acrolein | 20 | 19,300 | A |
| Acrylic acid | 1.04 | 4,210 | A |
| Allyl alcohol | 3.5 | 13,800 | A |
| Allyl chloride | 25 | 17,900 | A |
| Allyl isothiocyanate | .008[b] | 901,000 | C |
| Ammonia | 55 | 167,300 | A |
| Amyl acetate | 20 | 2,500 | A |
| Amyl alcohol | 1.0 | 3,700 | A |
| Anethole | .002[b] | 4,400 | A |
| Aniline | 0.8[b] | 400 | A |
| Benzaldenyde | 0.005[b] | 22,000 | C |
| p-benzoquinone | 0.15 | 790 | A |
| Benzylchloride | 0.05[b] | 28,000 | C |
| Benzyl sulfide | 0.0021 | | C |
| Bornylacetate | 0.0075[b] | 1,700 | A |
| Bromine | 0.047 | | A |
| Bromoacetone | | | * |
| 1,3-butadiene | 1.3 | 2,530 | A |
| n-butane | 5000[b] | 480 | A |
| n-butanol | 2.0 | 2,630 | A |
| sec butanol | 0.56 | 28,179 | A |
| tert butanol | 0.73[b] | 55,900 | A |
| n-butylacetate | 0.037 | 1,200 | A |

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TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| n-butylamine | 0.24 | 395,000 | B |
| Butyl cellosolve acetate | 0.48 | 2,729 | A |
| α-butylene | 0.069[b] | 43,480,000 | B |
| β-butylene | 0.60[b] | 3,333,000 | A |
| 1,2-butyleneoxide | 0.71 | 260,563 | A |
| Butyl glycolether | 0.48 | 1,650 | A |
| n-butyl chloride | 16.7 | 6,377 | A |
| n-butyl ether | 0.47 | | A |
| n-butyl formate | 20[c] | | A |
| n-butyl mercaptan | .0008 | 49,000,000 | E |
| n-butyl sulfide | 0.002 | 658,000 | * |
| t-butyl mercaptan | .00008 | | * |
| Butyraldehyde | 0.039 | 2,984,615 | B |
| n-butyric acid | 0.02[b] | 50,000 | * |
| Camphor | 16[b] | 41 | A |
| Caproic acid | .006[b] | 43,900 | D |
| ε-caprolactam | .063[b] | 20 | A |
| Caprylic acid | .008[b] | 164,500 | D |
| Carbon disulfide | 8[b] | 44,430 | A |
| Carbon tetrachloride | 250 | 540 | A |
| Carbitol acetate | 0.263 | 250 | A |
| Carvacrol | .0022[b] | 6,600 | A |
| Cellosolve acetate | 0.25 | 6,315 | A |
| Cellosolve solvent | 1.3 | 3,800 | A |
| Chloral | .047 | 980,000 | D |
| Chlorine | 0.314 | | A |
| α-chloroacetophenome | .016[b] | 330 | A |
| Chlorobenzene | 0.22[b] | 52,600 | C |
| Chlorobromomethane | 408[b] | 350 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Chloropicrin | 1.1[b] | 22,200 | A |
| Chloroprene | 0.11[b] | 2,390,000 | D |
| Cinnamaldehyde | .0025[b] | 53,000 | * |
| Citronellal (3-7-dimethyl-6-octenal) | | | B |
| o-cresol | 0.7[b] | 60 | A |
| m-cresol | 0.27[b] | 80 | A |
| p-cresol | 0.20[b] | 260 | A |
| Croton aldehyde | 0.20[b] | 125,000 | B |
| Cyanogen chloride | 1.0[b] | 1,300,000 | A |
| Cyclonexane | 0.50[c] | 203,000 | A |
| Cyclohexanol | 0.05[b] | 26,300 | D |
| Cyclonexanone | 0.24 | 21,900 | B |
| 1-decanol | 0.0063[b] | 31,000 | D |
| 1-decene | 0.0113[b] | 23,000,000 | D |
| Diacetone alconol | 1.7 | 774 | A |
| di-N-butylamine | 0.48 | 5,479 | A |
| o-dichlorobenzene | 50[b] | 26 | A |
| p-dichlorobenzene | 30[b] | 26 | A |
| 1,1-dichloroethylene | 500[b] | 1,300 | A |
| 2,2-dichloroethyl ether | 15[b] | 60 | A |
| 1,2-dichloropropane | 50[b] | 1,100 | A |
| a-dicyclopentadiene | 0.02 | 440,500 | B |
| Diethylamine | 0.06 | 4,250,000 | B |
| 2-diethylaminoethanol | 0.04 | 46,000 | D |
| Diethylselenide | 0.001[b] | 3,200,000 | D |
| Diethyl succinate | 0.015[b] | 800 | A |
| Diethyl sulfide | 0.0042[b] | 14,000,000 | D |
| Diethyl ketone | 9[b] | 1,900 | A |

TABLE A-1.  (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Diglycol | 1.1 | 120 | A |
| Diisobutyl carbinol | .16[b] | 8,187 | A |
| Diisobutyl ketone | .31 | 7,215 | A |
| Diisopropylamine | 0.70[b] | 108,000 | A |
| Diisopropyl ether | 0.053 | 3,227,400 | B |
| N,N-dimethylacetamide | 46.2[b] | 37 | A |
| Dimethylamine | 0.6[b] | 280,000 | A |
| Dimethylethanolamine | 0.045 | 292,400 | D |
| N,N-dimethyl formamide | 0.046[b] | 77,200 | B |
| Dimethylsulfide | 0.003 | 184,000,000 | D |
| 1,3-dioxolane | 128.0 | 804 | A |
| Diphenylether | 0.1[b] | 263 | A |
| Diphenylsulfide | .0021[b] | 31,000 | D |
| Dipnosgene | 1.1[b] | 11,906 | A |
| di-N-propylamine | 0.1 | 395,000 | D |
| 1-dodecanol | .0071[b] | 1,800 | A |
| Enonthic acid | .015[b] | 900 | A |
| Ethane | 1520[b] | 25,300 | A |
| Ethanol | 350[b] | 11 | A |
| Ethanolamine | 4[c] | 130 | A |
| 2-ethoxy-3,4-dihydropyran | 0.6 | 10,900 | A |
| Ethylacetate | 13.2 | 7,300 | A |
| Ethylacrylate | .00036 | 106,000,000 | B |
| Ethylamine | 0.83 | 634,000 | A |
| Ethyl hexanoate | 0.0052 | 760,000 | D |
| Ethylhexyl acetate | 0.21 | | B |
| Ethylidene norbornene | 0.073 | 75,600 | D |
| Ethylisoamylketone | 5 | 660 | A |
| Ethyl isovalerate | 0.12 | 88,000 | D |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| 2-ethyl-1-butanol | 0.77 | 3,075 | A |
| Ethylbutyrate | 0.0075 | 1,982,000 | B |
| Ethylene | 700 | 57,100 | A |
| Ethylene diamine | 11.2 | 1,057 | A |
| Ethylene bromide | 26 | 550 | A |
| Ethylene dichloride | 40 | 2,037 | A |
| Ethylene glycol | 25[b] | 3 | A |
| Ethyl glycol | 1.33 | 3,760 | A |
| Ethyl glycolacetate | 0.250 | 6,300 | A |
| Ethyleneimine | 2.0 | 105,300 | A |
| Ethylether | .33[b] | 1,940,000 | A |
| 2-ethyl-1-hexanol | 0.138 | 480 | A |
| 2-ethylhexylacrylate | 0.18 | 7,310 | A |
| Ethyl mercaptan | 0.0005[b] | 289,500,000 | B |
| N-ethylmorpholine | 0.25 | 32,100 | A |
| Ethyl pelargonate | 0.00012 | 109,000 | D |
| Ethyl silicate | 7.2 | 182 | A |
| Ethyl-n-valerate | 0.049[b] | 178,000 | D |
| Eugenol | 0.0035[b] | 37,600 | D |
| Formic acid | 21 | 2,200 | A |
| Furfural | 0.25[b] | 5,260 | A |
| Furfuryl alochol | 8 | 5,260 | A |
| Glycoldiacetate | 0.312 | 1,687 | A |
| Heptane | 223[b] | 200 | A |
| 1-heptanol | .057 | 23,100 | D |
| 2-heptanone | .020 | 171,000 | D |
| Hexachlorocyclopentadiene | 0.15 | 700 | A |
| n-hexanol | .090[b] | 14,300 | D |
| sec hexaylacetate | 0.40 | 12,500 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Hexylene glycol | 50 | 2 | A |
| Hydrochloric acid gas | 10.0 | | A |
| Hydrocinnamic alcohol | 0.00026[b] | 253,000 | D |
| Hydrogen cyanide | 5.0[b] | 163,000 | A |
| Hydrogen sulfide | 1.1[b] | 17,000,000 | A |
| α-ionone | 0.0000125[b] | 1,050,000 | D |
| Isoamyl acetate | .015[b] | 526,000 | * |
| Isoamyl alcohol | 1.0 | 3,026 | A |
| Isoamyl isovalerate | .001[b] | 1,050,000 | D |
| Isoamyl sulfide | .0004 | 1,640,000 | D |
| Isobutane | 1.2[b] | 3,000,000 | A |
| Isobutanol | 2.05 | 5,131 | A |
| Isobutene | 0.56[b] | 4,640,000 | A |
| Isobutyl acetate | 0.50 | 34,200 | A |
| Isobutyl acrylate | 0.012 | 525,000 | D |
| Isobutyl cellosolve | 0.191 | 34,400 | D |
| Isobutyraldehyde | 0.236 | 947,000 | B |
| Isodecanol | 0.042 | 300 | A |
| Isopentanoic acid | 0.026 | 9,600 | A |
| Isophorone | 0.54 | 900 | A |
| Isopropanol | 28.2 | 1,540 | A |
| Isopropylacetate | 0.97 | 57,000 | A |
| Isopropylamine | 0.95 | 637,000 | A |
| Isopropylbenzene | 0.047 | 89,600 | C |
| Isopropyl mercaptan | 0.00025[b] | 1,052,000,000 | D |
| Isovaleric acid | 0.0018 | 365,500 | D |
| Linalylacetate | 0.0016 | 66,000 | D |
| Maleic anhydride | 0.425[b] | 0.2 | A |
| Menthol | 1.3[b] | 100 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Mesityloxide | .051 | 224,460 | B |
| Methacrylonotrile | 1.8[b] | 72,000 | A |
| Methanol | 53.3 | 2,262 | A |
| Methylacetate | .207[b] | 1,080 | A |
| Methylamine | 3.3[b] | 940,000 | A |
| Methylamylalcohol | 0.52 | 12,634 | A |
| Methylanthranilate | .00065[b] | 101,000 | D |
| 2-methyl-2-butanol | 0.23 | 17,130 | D |
| Methyl butyrate | 0.0026[b] | 19,200,000 | B |
| Metnyl chloride | 10.8[b] | 200,000 · | A |
| 4-methylcyclohexanol | 500[b] | 3,700 | A |
| Methylene chloride | 150[b] | 3,060 | A |
| N-methylethanolamine | 3.4 | 400 | A |
| Methylethyl ketone | 6.0 | 17,000 | A |
| 2-methyl 5-ethylpyridine | 0.010 | 137,000 | D |
| Methyl formate | 2,000 | 300 | A |
| Methyl glycol | 0.40 | 20,400 | A |
| Methyl glycolacetate | 0.64 | 14,400 | A |
| Methyl isoamyl alcohol | 0.20 | 6,600 | A |
| Methyl isoamyl ketone | 0.070 | 75,142 | D |
| Methyl isobutyl ketone | 0.28 | 28,195 | B |
| Methyl isopropenyl ketone | 0.29[b] | 184,000 | D |
| Methyl mercaptan | .020[b] | 33,300,000 | B |
| Methyl methacrylate | .34 | 108,000 | A |
| 2-methyl pentaldehyde | 0.136 | 131,500 | D |
| 2-methyl 1-pentanol | 0.082 | 24,000 | D |
| Methyl salicylate | 0.00058[b] | 113,400 | * |
| a-methyl styrene | 0.156 | 19,400 | C |
| Monochloroacetic acid | 0.045[b] | 1,460 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Monochlorobenzene | 0.21 | 52,600 | C |
| Morpholine | 0.14 | 75,200 | B |
| Naphthalene | 0.027 | 2,400 | A |
| Nitrobenzene | 1 | 200 | A |
| Nitromethane | 100 | 460 | A |
| 1-nitropropane | 300 | 40 | A |
| n-nonane | 0.43 | 9,800 | A |
| n-octane | 150 | 100 | A |
| 1-octanol | 0.002[b] | 33,000 | D |
| 2-octanol | 0.00026[b] | 506,000 | * |
| 2-octanone | 248 | 4 | A |
| n-octylacetate | 0.21 | 2,500 | A |
| Paracresol | 0.001 | | D |
| Pelargonic acid | .00084[b] | 164,000 | * |
| n-pentane | 990 | 570 | A |
| 2,4-pentanedione | 0.024 | 750,000 | D |
| 1-pentanol | 1.0 | 3,700 | A |
| 2-pentanone | 8 | 1,973 | A |
| 1-pentene | .0021[b] | 376,000,000 | B |
| Phenacyl bromide | | | D |
| Phenol | 0.65[b] | 404 | A |
| Phenyl isocyanide | 0.001 | 3,950,000 | D |
| Phenyl mercaptan | 0.0002[b] | 94 | A |
| Phosgene | 0.25 | 6,400,000 | B |
| Phosphine | 0.021 | | B |
| α-picoline | 0.046 | 228,800 | D |
| α-pinene | 0.0114[b] | 469,000 | D |
| Piperonal | 0.0043[b] | 0.10 | A |
| Propane | 20,000 | 425 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| n-propanol | 0.13 | 146,760 | B |
| Propene oxide | 35.0 | 16,600 | A |
| Propionaldehyde | 0.08 | 3,865,000 | B |
| Propionic acid | 0.034 | 112,200 | * |
| n-propylacetate | 0.15 | 219,300 | B |
| Propylene | 67.6 | 14,792 | A |
| Propylene diamine | 0.067 | 184,600 | B |
| n-propyl mercaptan | 0.00075 | 263,000,000 | D |
| Pyridine | 0.1 | 184,200 | * |
| Skatole | 0.00021 | 30,000 | * |
| Styrene | 0.15 | 43,900 | B |
| Styrene oxide | 0.40 | 1,000 | A |
| Sulfur dichloride | 0.001 | | * |
| Sulfur dioxide | 0.47 | | A |
| 1,1,2,2-tetrachloroethane | 3 | 2,193 | A |
| 1,1,2,2-tetrachloroethylene | 50[b] | 370 | A |
| Tetraethyl o-silicate | 7.2 | 193 | A |
| Tetrahydrofuran | 30[b] | 5,800 | A |
| 1,2,3,5-tetramethylbenzene | .003[b] | 136,000 | C |
| Thiophane | | | * |
| Thymol | 0.00085[b] | 155,000 | * |
| Toluene | 0.4[b] | 33 | A |
| o-tolyl mercaptan | 0.0027[b] | 39,000 | D |
| 1,1,1-trichloroethane | 400[b] | 330 | A |
| Trichloroethylene | 80[b] | 1,000 | A |
| Trichlorofluoromethane | 209 | 4,325 | A |
| 1,1,2-trichloro,1,2,2-trifluoroethane | 135 | 2,630 | A |

TABLE A-1. (Continued)

| Compound | Odor Recognition Threshold, ppm | Odor Index (20 C) | Disposition Code[a] |
|---|---|---|---|
| Triethylamine | 0.28 | 234,000 | B |
| Trimethylamine | 3.85[a] | 493,000 | A |
| n-undecane | 0.11[b] | 8,400 | A |
| Valeric acid | 0.0006[b] | 329,000 | * |
| Vanilline | 0.000016[b] | 822,000 | D |
| Vinylacetate | 0.55 | 198,500 | A |
| Vinyl-2-pyridine | 0.30[b] | 6,600 | A |
| o-xylene | 0.27 | 24,360 | B |
| m-xylene | 3.7[b] | 2,100 | A |
| p-xylene | 0.47 | 18,200 | A |
| Xylidine | 0.0048[b] | 82,000 | D |

(a) Odor disposition code (A: eliminated because of low odor index or high odor threshold; B: eliminated because of low boiling point or flash point; C: carcinogen or suspected carcinogen; D: eliminated because of weak or pleasant odor, or member of a class already selected, or due to missing information *: selected for further investigation).

(b) 50 percent recognition threshold.

(c) Absolute odor threshold (50 percent).

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APPENDIX B

TOXICITY OF CANDIDATE TEST COMPOUNDS

APPENDIX B


TOXICITY OF CANDIDATE TEST COMPOUNDS


## Exposure Characterization

Prior to presenting the toxicity data obtained from the literature
for the candidate compounds, it is worth considering the potential exposure
the subjects will experience.  For this assessment, we will assume that the
subject will experience each of the challenge concentrations for one minute
each.  This would be the case for a subject who cannot detect the vapor's
odor and inhales the test vapor for one minute at every concentration level
before selecting one of the available gas streams as the one containing the
odorant.  The published values of odor threshold are presented in the
following table for each compound.  The maximum concentration to which the
subjects may be exposed (for up to one minute) are listed as 27 times greater
than the published odor threshold value.  The absorbed mass is calculated
assuming total absorption of the compound at all concentrations used and
assuming a breathing rate of 12.5 lpm for the subject.  The final figure in
the column is the dosage based on a body weight of 70 kg for the subject.
This column represents the worst case dosage which can be achieved in this
testing.  It should also be noted that this a one-time exposure for the
subjects.

| Compound | Odor Threshold, ng/1 | Maximum Conc, ng/1 | Absorbed Mass, µg | Dosage, µg/kg |
|---|---|---|---|---|
| t-butyl mercaptan | 0.3 | 8.1 | 0.15 | 0.002 |
| Isoamyl acetate | 81 | 2190 | 41 | 0.6 |
| Methyl salicylate | 3.7 | 100 | 1.9 | 0.03 |
| Nonanoic acid | 5.5 | 148 | 2.8 | 0.04 |
| Skatole | 1.2 | 32 | 0.61 | 0.01 |

The following sections of this Appendix present information on the
use and occurrence of the candidate compounds as well as a review of the
available toxicologic information.

## Methyl Salicylate

### I. Use and Occurrence

Methyl salicylate occurs naturally in wintergreen oil, Gaultheria procumbers and birch, tuberose, Dianthus caryophyllus, Acia cavenia and ylang-ylang. It is also present in many fruit juices including cherry, apple, and raspberry (Fenarole's Handbook of Flavor Ingredients, 1975). The oil is steam-distilled from leaves and allowed to macerate for several hours. Distillation for 5 to 6 hours yields about 0.7% oil. Methyl salicylate can be prepared synthetically by the esterification of salicycylic acid with methanole using sulfuric acid as the catalyst. The product is about 99% pure (Merck Index, 1976).

In public use since the 1930s, methyl salicylate is the most important commercial derivative of salicylic acid other than aspirin (Erickson, 1982). Its use in the fragrance industry amounts to about 90,000 lb/year (Opdyke, 1978). In 1975, 2,330 metric tons were produced in the United States (Erickson, 1982). Methyl salicylate is also used as a solvent for cellulose derivatives, in insecticides, in polishes, and in printing and copying inks (Hawley, 1971; Erickson, 1982). As a pharmaceutical product, it is used in topical ointments and linaments for lumbar and sciatic pain relief and for rheumatism. It is also used as a UV-absorber in suntan lotion (Erickson, 1982). It is also used as a local analegisic for veterinary medicine. As an artificial flavoring, methyl salicylate is found as an important ingredient in toothpaste, candy, chewing gum, ice cream, baked goods, non-alcoholic beverages, syrups, and pharmaceuticals. Other commercial applications include its use as a dye carrier for synthetic fibers, UV light stabilizer, and in acrylic resins. Typical concentrations (percentages) in final products are as follows (Opdyke, 1978):

|         | Soap | Detergent | Creams, lotions | Perfume |
|---------|------|-----------|-----------------|---------|
| Usual   | 0.03 | 0.003     | 0.01            | 0.05    |
| Maximum | 0.3  | 0.03      | 0.15            | 0.8     |

Methyl salicylate has been designated as GRAS (generally regarded as safe) by FEMA (1965). The Council of Europe has recommended an acceptable

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daily intake (ADI) for methyl salicylate of 0.5 mg/kg (Opdyke, 1978). The Joint FAO/WHO Expert Committee on Food Additives (1967) has assigned it an unconditional ADI of 0 to 0.5 mg/kg. Also, liquid preparations containing more than 5% (w/w) methyl salicylate, except for those packaged in pressurized spray containers, are required to be packaged in accordance with the Poison Prevention Act of 1970 (Opdyke, 1978).

## II. Information Resources

Toxicity and human health effects information on methyl salicylate were obtained from a number of resources, including on-line computer data bases.

An overview of information on acute toxicity, carcinogenicity, mutagenicity, reproductive toxicity/teratogeniticy, and human exposure was obtained from Opdyke (1978). An attempt to obtain more specific information on the carcinogenic potential on methyl salicylate was made with the summary reports of the National Toxicology Program (NTP-DHHS, 1985), the annual plan of the National Toxicology Program (NTP-DHHS, 1986), surveys of the National Cancer Institute (NCI, 1979-80), NIOSH's (1984) Registry of Toxic Effects of Chemical Substances (RTECS) and Sax (1984). Specific mutagenicity information was sought from RTECS and Sax. In addition, information on the mutagenic properties of methyl salicylate were checked with a hard copy of the computer data base GENETOX, published in Palajda and Rosenkranz (1985). Principal sources of acute toxicity information included Opdyke, RTECS, Sax and Patty's (1981) with teratogenicity information obtained from Sax, RTECS, Shepard (1986) and Schardein (1985). Toxicity information was also obtained from the U.S. Army Protocol for Field Entry/Exit Test (U.S. Army CRDEC, 1986). Information on occupational exposure guidelines was sought from the NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards (1981) and the Documentation of the American Conference of Governmental Industrial Hygienists (1986). Computerized data bases accessed for health and toxicity information included MEDLINE, TOXLINE, (TOXBACK 76, TOXBACK 65), NTIS, and the Hazardous Substance Data Base (TOXNET).

## III. Characterization of Exposure

Methyl salicylate passed a preliminary toxicological screening step as a candidate substance for replacing isoamyl acetate as a qualitative fit test stimulant. If selected as an appropriate substitute, methyl salicylate would be used on a limited basis in this project for odor threshold determinations and fit testing.

The anticipated uses of the substitute will be identical to those currently employed by the Army for isomyl acetate as a simulant for fit testing. Therefore, human exposures through the respiratory and dermal routes are likely to occur but are expected to be of limited dose and duration. Oral exposures are not anticipated. Based on these projected characteristics of exposure, the toxicity of the candidate substance is ideally ascertained for the respiratory and dermal routes of exposure. The available toxicity information is presented below.

## IV. Toxicity Review

According to Opdyke (1978) who has reviewed the toxicity of methyl salicylate, this substance has been tested on several occasions for acute toxicity. In mice, the $LD_{50}$ is 1,110 mg/kg and in rats values of 887 and 1,250 mg/kg have been observed. Oral administration of 0.5 ml to rats by gavage has caused slight redness and irritation of the stomach mucosa. Dosages of 0.6 to 4.7 g/kg by intubation to the stomach and duodenum of dogs caused primary nausea, vomiting, intense hypernea, excitation of the central nervous system, diarrhea and death. A lethal oral dose in children is considered to be approximately 4 to 8 ml. Signs of poisoning usually include excitation of central nervous system, abnormally rapid breathing, fever, high blood pressure, increased heartbeat, generalized convulsions and coma (Opdyke, 1978).

The acute dermal $LD_{50}$ in rabbits exceeds 5 g/kg. Pecutaneous absorption occurs because methyl salicylate has been detected in the muscle one hour after administration to skin of rabbits (Opdyke, 1978). Skin absorption has also been noted after application to rats at several pH values. One maximization test indicated that methyl salicylate does not

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cause sensitization in humans when administered at 8% in petrolatum; however, it is regarded as a moderate skin irritant. Necrosis and intradermal and subcutaneous hemorrhage were seen in one study in which the material was applied to shaved rabbit skin in concentrations as low as 1%. Guinea pigs may be more sensitive than rabbits. In petrolatum at concentrations of 8%, methyl salicylate produced no skin irritation after a 48-hour closed-patch test on human subjects (Opdyke, 1978). Material Safety Data Sheets from Monsanto and Tenneco warn that methyl salicylate is an eye irritant.

Feeding studies have been undertaken with methyl salicylate in which concentrations of 0.1, 0.5, 1.0 or 2% were administered in the diet of rats for 2 years. All rats in the 2% group died within 50 weeks and growth retardation and cancellous bone was found in the 1 and 2% groups. Based on the results in another 2-year study with rats, cancellous bone and other effects do not occur at dosages below 0.21% in the diet.

One cancer bioassay has been performed in which equivocal evidence of carcinogenicity was reported following intraperitoneal injections to mice of 2,400 mg/kg over an 8-week period (NIOSH, 1984).

Opdyke (1978) also reviewed the results of one inhalation study involving twenty, 7-hour exposures to rats with methyl salicylate vapour. No evidence of toxicity was found following exposures to 120 ppm.

Schardein (1985) has summarized the teratogenic effects of numerous chemicals in commerce and reported that there is evidence of teratogenicity in mice, rabbits and rats. Opdyke (1978) reviewed several studies indicating that methyl salicylate causes abnormalities in rats following intraperitoneal or subcutaneous injection. Teratogenic effects were not seen in a three generation dietary study, but there were decreases in average litter size and average numbers of live-born progeny at dietary doses of 3,000 and 5,000 ppm. There is some evidence that women who have delivered babies with birth defects may have used salicylate preparations. This finding may be relevant to this substance because methyl salicylate is metabolized to salicylic acid (Opdyke, 1978).

# Skatole

## I. Use and Occurrence

Skatole, also known as 3-methylindole, occurs naturally in various species of Nectandra and in the woods of Altis reticulosa and C. durandii (Uritcaceae). In animals, it can be found in feces and in the civet cat. Coal tar also contains natural levels of skatole.

In ruminants, 3-methylindole is the main ruminal fermentation product of L-tryptophan (Bray and Carlson, 1979). 3-methylindole is metabolized by mixed function oxidases (Bray and Kubow, 1985) and has been demonstrated as the cause of acute bovine pulmonary edema and emphysema after systemic exposure (see discussion below). 3-methylindole is also found in human feces in concentrations from 5 to 100 $\mu$g/g feces (personal communication, Dr. G. Yost).

Skatole can be produced synthetically from the phenylhydrazone of propionaldehyde or by cyclization of o-toluicides (Bedoukian, 1967). It has been in public use since the 1920's; its use in fragrances in the United States amounts to approximately 1,000 lb/yr (Opdyke, 1976). Skatole is also used in soaps, detergents, creams, and lotions and can be found in the following concentrations (percentages) of the final products (Opdyke, 1976):

|         | Soap  | Detergent | Cream, lotions | Perfume |
|---------|-------|-----------|----------------|---------|
| Usual   | 0.003 | 0.0003    | 0.0015         | 0.02    |
| Maximum | 0.03  | 0.003     | 0.01           | 0.1     |

Skatole has been designated as "generally regarded as safe" (GRAS) by FEMA (1965) and has been approved by the FDA for use in food. The Council of Europe (1974) listed skatole as an artificial flavoring (food additive) without hazard to public health at a level of 1 ppm.

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## II. Information Resources

A battery of information resources was brought to bear to obtain human health effects and toxicity information on skatole. These included widely-recognized texts and on-line computerized data bases.

An overview of toxicity information was obtained from Opdyke (1976). More specific information on the carcinogenic potential of skatole was sought from the summary report of the National Toxicology Program (NTP-HDDS, 1985), the annual plan of the National Toxicology Program (NTP-DHHS, 1986), surveys of the National Cancer Institute (NCI, 1979-80), NIOSH's (1984) Registry of Toxic Effects of Chemical Substances (RTECS), and Sax (1984). Reproductive toxicity/teratogenic potential was searched in RTECS, Sax, Shepard (1986), and Schardein (1985). Mutagenicity information was searched in Sax, RTECS, and a hard copy of the computerized data base GENETOX, published in Palajda and Rosenkranz (1985). Principal sources of acute toxicity were Opdyke, RTECS, Sax, and Patty's (1981).

For occupational exposure limit information, a variety of resources were tapped. The existence of guidelines in the United States was checked in NIOSH/OSHA's Occupational Health Guidelines for Chemical Hazards (1981) and in the Documentation of the American Conference of Governmental Industrial Hygienists (1986). In addition, based on a statement from the Opdyke (1976) review report, occupational exposure limits for the United Kingdom were checked to determine if a guideline for skatole exists. Further, the Alberta (Canada) Worker's Health, Safety, and Compensation Board was contacted to see if Canada has promulgated an occupational exposure guideline for skatole. Kodak, a manufacturer of skatole, was also contacted to discuss occupational exposure guidelines.

Several on-line computer databases were accessed to obtain information on skatole. These included MEDLINE, TOXLINE, (TOXBACK 76, TOXBACK 65), NTIS, and the Hazardous Substance Data Base (TOXNET). As a result of the TOXLINE search, several recent abstracts were found of studies on the pulmonary toxicity of skatole especially to bovines and other ruminants. Several of the veterinarians who conducted the studies were contacted by telephone to discuss the existence of any human exposure studies or any subchronic or chronic inhalation studies of which they might be aware.

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## III. Characterization of Exposure

Skatole passed a preliminary toxicological screening step as a candidate substance for replacing isoamyl acetate as a qualitative fit test stimulant. If selected as an appropriate substitute, skatole would be used on a limited basis in this project for odor threshold determinations and fit testing.

The anticipated uses of the substitute will be identical to those currently employed by the Army for isoamyl acetate as a simulant for fit testing. Therefore, human exposures through the respiratory and dermal routes are likely to occur but are expected to be of limited dose and duration. Oral exposures are not anticipated. Based on these projected characteristics of exposure, the toxicity of the candidate substance is ideally ascertained for the respiratory and dermal routes of exposure. The available toxicity information is presented below.

## IV. Toxicity Review

Skatole is regarded as a slightly toxic substance in which the acute oral $LD_{50}$ is 3,450 mg/kg in the rat and the dermal $LD_{50}$ in rabbits is greater than 5 g/kg (Opdyke, 1976). The intraperitoneal $LD_{50}$ in mice is 175 mg/kg. Single oral doses of 100 or 200 mg/kg have been given to mice resulting in pulmonary congestion in the 200 mg/kg group but no toxicity in the lower dose group.

Skatole is believed to be an intermediate involved in acute bovine pulmonary edema which occurs when cattle grazing on dry pasture are moved to lush green pasture. Ruminant microorganisms convert tryptophan (found in green pasture) to skatole (Hammond et al., 1979). Death from pulmonary edema and emphysema has occurred in cattle given 200 mg/kg orally and 60 mg/kg intravenously in proplyene glycol. Oral doses of 300 mg/kg have also caused diffuse pulmonary edema and death in goats.

Skatole has been applied full strength to intact or abraded rabbit skin for 24 hours under occlusion without evidence of irritation. Also, no irritation was seen in humans in which the material was administered in a 48-hour closed patch test at a 2% concentration in petrolatum. Sensitization was

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not observed in a maximization test involving 20 volunteers exposed to 2 percent skatole in petrolatum.

Only one inhalation study involving a mixture containing skatole has been conducted (Sandage, 1961). Male rats, mice and rhesus monkeys were exposed for 90 days to a mixture of indole (10.5 ppm), skatole (3.5 ppm), hydrogen sulfide (20 ppm) and methyl mercaptan (50 ppm). Effects such as anemia in all species, unspecified liver lesions in all species, and unspecified liver lesions in mice alone were observed. Eight of ten monkeys died for unknown reasons. The role of skatole, if any, in the effects seen in this 90-day study could not be ascertained. The investigator indicated that the concentrations selected for study were "industrial threshold limit values". However, currently there is no exposure limit enforced by OSHA, nor recommended by NIOSH, ACGIH, or another country for skatole.

# t-Butyl Mercaptan

## I. Use and Occurrence

The main commercial use of t-butyl mercaptan involves its use as an odorant for natural gas.

## II. Information Resources

A variety of references and data bases were accessed for health information on t-butyl mercaptan to assess its potential as a carcinogen, mutagen, teratogen and acute toxicant. Information on carcinogenic activity of t-butyl mercaptan was sought from the summary reports of the National Toxicology Program (NTP-DHHS, 1985), the annual plan of the National Toxicology Program (NTP-DHHS, 1986), surveys of the National Cancer Institute (NCA, 1979-80), NIOSH's (1984) Registry of Toxic Effects of Chemical Substances (RTECS), and Sax (1984). Reproductivity toxicity and teratogenicity were ascertained through information in RTECS, Sax, Schardein (1985), and Shepard (1986). For mutagenicity information, RTECS and Sax were consulted. Also, information on mutagenic properties of t-butyl mercaptan were checked with a hard copy of the computer data base GENETOX, published in Palajda and Rosenkranz (1985). Principal sources of acute toxicity information were Opdyke, RTECS, Sax, and Patty's (1981). For occupational exposure guidelines, the NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards (1981) and the Documentation of the American Conference of Governmental Industrial Hygienists (1986) were consulted.

Computerized data bases accessed for health information on t-butyl mercaptan included MEDLINE, TOXLINE (TOXBACK 76, TOXBACK 65), NTIS, and the Hazardous Substance Data Base (TOXNET).

## III. Characterization of Exposure

T-butyl mercaptan passed a preliminary toxicological screening step as a candidate substance for replacing isoamyl acetate as a qualitative fit test stimulant.  If selected as an appropriate substitute, t-butyl mercaptan would be used on a limited basis in this project for odor threshold determinations and fit testing.

The anticipated uses of the substitute will be identical to those currently employed by the Army for isoamyl acetate as a simulant for fit testing.  Therefore, human exposures through the respiratory and dermal routes are likely to occur but are expected to be of limited dose and duration.  Oral exposures are not anticipated.  Based on these projected characteristics of exposure, the toxicity of the candidate substance is ideally ascertained for the respiratory and dermal routes of exposure.  The available toxicity information is presented in the Section IV.

## IV. Toxicity Review

The oral $LD_{50}$ of t-butyl mercaptan in the rat is 4,729 mg/kg indicating it is slightly toxic by the oral route and exposure (Sandmeyer, 1981). By the inhalation route for a 4-hour exposure period, the $LC_{50}$ is 22,200 ppm for the rat and 16,500 ppm for the mouse (Patty's, 1981).  This substance is an eye irritant in the rabbit following administration of 84 mg (NIOSH, 1984).

# Nonanoic Acid

## I. Use and Occurrence

Nonanoic acid, also known as pelargonic acid, has been reported to occur naturally in various oils such as those of rose, geranium, arris, Litsea cubeta, Artemisia arborescens, hops, Chamaecyparis pisifera, Eremocitrus glauca, and French lavender and in oak moss (Opdyke, 1978). It can be synthetically prepared by oxidation of oleic acid (Arctander, 1969).

Nonanoic acid has been in use since the 1930's in the perfume, soup, and detergent industries. It is also found in certain creams and lotions. Further, nonanoic acid is used in the production of hydrotropic salts and the manufacture of lacquers and plastics. It is also used as an artificial flavoring and odor, as a gasoline additive, in the manufacture of esters for turbojet lubricants and as a flotation agent (Hawley, 1971; Windholz et al., 1983). Typical concentrations (percentages) on nonanoic acid found in final products are as follows (Opdyke, 1978):

|         | Soap | Detergent | Creams, lotions | Perfumes |
|---------|------|-----------|-----------------|----------|
| Usual   | 0.01 | 0.001     | 0.005           | 0.04     |
| Maximum | 0.1  | 0.01      | 0.01            | 0.2      |

## II. Information Resources

Several information resources were accessed to obtain toxicity and human health effects information on nonanoic acid. These resources included several large texts and computerized data bases.

An overview of toxicity information was obtained from Opdyke (1978). This series of articles provides limited information on carcinogenicity, mutagenicity, teratogenicity, and human exposure, if available. More specific carcinogenic information was sought from the summary reports of the National Toxicology Program (NTP-DHHS, 1985), the annual plan of the National Toxicology Program (NTP-DHHS, 1986), surveys of the National Cancer Institute (NCI, 1979-80), NIOSH's (1984) Registry of Toxic Effects of Chemical Substances (RTECS) and Sax (1984). An attempt to obtain additional mutagenicity

information from RTECS, Sax, and a hard copy of the computerized data base
GENETOX, published in Palajda and Rosenkranz (1985) was made. Reproductive
toxicity/teratogenicity information was sought in RTECS, Sax, Shepard (1985)
and Schardein (1986). Principal sources of acute toxicity were Opdyke,
RTECS, Sax, and Patty's (1981).

For information on occupational exposure limits, NIOSH/OSHA's
Occupational Health Guidelines for Chemical Hazards (1981) and the Documenta-
tion of the American Conference of Governmental Industrial Hygienists (1986)
were consulted.

Computerized data bases accessed for toxicity and human health
effects information included MEDLINE, TOXLINE (TOXBACK 76, TOXBACK 65), NTIS,
and the Hazardous Substances Data Base (TOXNET).

## III. Characterization of Exposure

Nonanoic acid passed a preliminary toxicological screening step as a
candidate substance for replacing isoamyl acetate as a qualitative fit test
stimulant. If selected as an appropriate substitute, nonanoic acid would be
used on a limited basis in this project for odor threshold determinations and
fit testing.

The anticipated uses of the substitute will be identical to those
currently employed by the Army for isoamyl acetate as a simulant for fit
testing. Therefore, human exposures through the respiratory and dermal
routes are likely to occur but are expected to be of limited dose and
duration. Oral exposures are not anticipated. Based on these projected
characteristics of exposure, the toxicity of the candidate substance is
ideally ascertained for the respiratory and dermal routes of exposure. The
available toxicity information is presented in the following section.

## IV. Toxicity Review

Nonanoic acid is regarded as slightly toxic when exposures are
through the oral route. This substance has an oral $LD_{50}$ of 3,200 mg/kg in
the rat, but is considerably more toxic to mice when administered intrave-
nously ($LD_{50}$ = 244 mg/kg) in an emulsion based on cottonseed oil, emul-

sifiers, and buffer. Acute dermal toxicity is not an important considera-
tion, because the LD$_{50}$ in rabbits is greater than 5 g/kg (Opdyke, 1978).
Nonanoic acid has produced severe skin irritation in the guinea pig, but it
was moderately irritating to abraded rabbit skin applied for 24 hours under
occulusion. Human testing for skin irritation has been negative when
nonanoic acid was applied in petrolatum in 48-hour closed patch test.
However, signs of skin irritancy (erythema) were seen in seven of ten human
subjects after seven days of exposure in which the substance was administered
daily under occlusive patches (Opdyke, 1978). Nonanoic acid is a severe eye
irritant in the rabbit following exposure to 91 mg (NIOSH, 1984). Evidence
of the substance's capacity to cause sensitization is sketchy, but one study
involving 25 volunteers exposed to nonanoic acid in 12% petrolatum was
negative.

# Isoamyl Acetate

## I. Use and Occurrence

Isoamyl acetate occurs naturally in a variety of fruits including apples, bananas, cocoa beans, grapes, peaches, pears, pineapples, and strawberries. It has also been found in cognac (FEMA, 1974). This substance has been in public use since before the turn of the century, and it is a component of perfumes, creams, soaps, and lotions. Use in fragrances in the United States amounts to about 10,000 lb per year (Opdyke, 1975). Typical concentrations (percentages) in final products are as follows (Opdyke, 1975):

|         | Soap | Detergent | Creams/Lotions | Perfume |
|---------|------|-----------|----------------|---------|
| Usual   | 0.05 | 0.005     | 0.003          | 0.05    |
| Maximum | 0.2  | 0.02      | 0.02           | 0.3     |

In addition, isoamyl acetate is widely used by industrial hygienists to conduct fit tests with respiratory protection equipment due to its strong, banana-like odor.

Isoamyl acetate has been granted GRAS (generally regarded as safe) status by FEMA (1965) and is approved by the FDA for food use (21 CFR 172.515). The Council of Europe (1974) has recommended an ADI (acceptable daily intake) level of 1 mg/kg.

## II. Information Resources

An overview of the acute oral and dermal toxicity, and human exposure data for isoamyl acetate was obtained from Opdyke (1975). In addition, Sax (1984), NIOSH's Registry of Toxic Effects of Chemicals (NIOSH, 1984) and Patty's (1983) were referenced for acute toxicity information. Specific information on the carcinogenic potential of isoamyl acetate was ascertained through the Summary Reports of the National Toxicology Program (NTP-DHHS, 1985), the Annual Plan of the National Toxicology Program (NTP-DHHS, 1985), the surveys of the National Cancer Institute (NIH/NCI, 1984), RTECS, and Sax. Mutagenicity information was sought in RTECS, Sax, a

hardcopy of the computer database GENETOX published in Palajda and Rosenkranz (1985). Teratogenicity and reproductive toxicity data was searched in RTECS, Sax, Shepard (1986), and Schardein (1985). Information on occupational exposure guidelines was obtained from the NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards (1981) and the Documentation of the American Conference of Governmental Industrial Hygienists (1986).

III. Characterization of Exposure

Isoamyl acetate is frequently used by industrial hygienists in qualitative fit testing of respirators. As outlined in its lead standard, the Occupational Health and Safety Administration (OSHA) specifies the use of isoamyl acetate as one of the three allowable substances for qualitative fit protocols permissible for compliance with the standard (29 CFR 1910.1025, Appendix D). The U.S. Army also uses isoamyl acetate for qualitative fit tests of face masks, such as the M17 respirator (U.S. Army Technical Manual 3-4340-279-20 ANDT).

IV. Toxicity Review

The acute oral $LD_{50}$ of isoamyl acetate in rabbits is 7,422 mg/kg (Munch, 1972). The oral $LD_{50}$ in rats is also high, exceeding 5 g/kg (Moreno, 1973). The oral $LD_{50}$ for rabbits, administered by stomach tube, is 57 mmol/kg (Munch, 1972).

One inhalation study reported feline death when cats were exposed to 7,200 ppm isoamyl acetate for 24 hours (Lehman and Flury, 1943). Another inhalation study by Flury and Wirth (1933) yielded an $LC_{Lo}$ for cats of 6,583 ppm, while narcotic effects were noted in cats exposed for 6 hours to 2,800 ppm.

The acute dermal $LD_{50}$ of isoamyl acetate for rabbits exceeded 5 g/kg (Moreno, 1973). Isoamyl acetate was not irritating when applied full strength to intact or abraded rabbit skin under occlusion (Moreno, 1973). A 48-hour closed patch test on humans using an 8% concentration of isoamyl acetate in petrolatum produced no irritation (Kligman, 1973). Also, a maximization test carried out on 25 volunteers with isoamyl acetate at a

concentration of 8% in petrolatum produced no sensitization reactions (Kligman, 1973).

One study, testing the mutagenic potential of isoamyl acetate, found negative responses for induction of mitotic chromosomal malsegratation, mitotic recombination, and point mutation in diploid yeast (Zimmerman, et al., 1985).

Adverse effects have been recorded in humans following exposures to relatively high concentrations. Exposure of 950 ppm for 30 minutes resulted in moderate irritation to nose and eyes. Other symptoms included headache, mucous membrane irritation, conjunctiva, vertigo, palpitations, gastrointestinal disorders, anemia, and liver disorders. Inhalation of 200 ppm has caused severe throat irritation, with slight throat discomfort at 100 ppm (Nelson, et al., 1943).

The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a TLV-TWA of 100 ppm and a STEL (short-term exposure level) of 125 ppm for worker exposures to isoamyl acetate. The current Occupational Safety and Health Administration (OSHA) standard is 100 ppm averaged over an eight-hour work shift (NIOSH/OSHA, 1981).

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